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**A U S T R A L I A**  
**Patents Act 1990**

**PROVISIONAL SPECIFICATION**

for the invention entitled:

**"A METHOD OF MODULATING PLANT PHYSIOLOGICAL  
PROCESSES AND GENETIC SEQUENCES USEFUL FOR SAME - II"**

The invention is described in the following statement:

- 1A -

## A METHOD FOR MODULATING PLANT PHYSIOLOGICAL PROCESSES AND GENETIC SEQUENCES USEFUL FOR SAME-II

### FIELD OF THE INVENTION

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The present invention relates generally to a method for modulating plant physiological processes such as but not limited to resistance to plant pathogens, senescence, cell growth and the shape of cells, tissues and organs. The method of the present invention is predicated in part on the manipulation of starch metabolism and/or cell expansion as a means for example, of inducing 10 resistance to plant pathogens and to modulate senescence or to alter cell growth or shape. In one particular embodiment, the present invention contemplates a method of modulating plant physiological processes by manipulating amylase production in plant cells. Another particular embodiment provides the manipulation of cell shape and/or cell expansion.

### 15 BACKGROUND OF THE INVENTION

Bibliographic details of the publications numerically referred to in this specification are collected at the end of the description.

20 Genetic engineering is now an integral part of strategies to develop varieties of plants with commercially useful traits. Transposons have played an important part in the genetic engineering of plants to provide *inter alia* tagged regions of plant genomes to facilitate the isolation of genes by recombinant DNA techniques as well as to identify important regions in plant genomes responsible for certain physiological processes.

25

The maize transposon *Activator (Ac)* and its derivative *Dissociation (Ds)* comprise one of the first transposon systems to be discovered (1,2) and was first used to clone genes by Fedoroff *et al* (3). The behaviour of *Ac* in maize has been studied extensively and excision occurs in both somatic and germline tissue. Studies have highlighted two important features of *Ac/Ds* for 30 tagging. First, the transposition frequency and second, the preference of *Ac/Ds* for transposition in linked sites.

The use of the *Ac/Ds* system has been hampered by the difficulty of data interpretation due, for example, to the high activity of *Ac* in certain plants and insertions at unlinked sites arising from multiple transpositions rather than by a single event from the T-DNA. This problem was addressed by Jones *et al* (4), Carroll *et al* (5) and others where a two component *Ac/Ds* system 5 was developed. In this system, the *Ds* elements were made by replacing the *Ac* transposase gene with a marker gene thereby rendering it non-autonomous. T-DNA regions of binary vectors were constructed by Carroll *et al* (5) and Scofield *et al* (6) carrying either a *Ds* element or a stabilised Activator transposase gene (*sAc*). The *Ds* element contained a reporter gene (eg. *nos:BAR*) which was shown to be inactivated on crossing with plants carrying the *sAc* (5). This 10 is referred to as transgene silencing. It has been shown that transgene silencing is a more general phenomenon in transgenic plants (7, 8, 9). Many different types of transgene silencing have now been reported in the literature and include: co-suppression of a transgene and a homologous endogenous plant gene (10), inactivation of ectopically located homologous transgenes in transgenic plants (7), the silencing of transgenes leading to resistance to virus infection (11) and 15 inactivation of transgenes inserted in maize transposons in transgenic tomato (5).

Gene silencing undoubtedly reflects mechanisms of great importance in the understanding of plant gene regulation. Other important mechanisms include anti-methylation sequences (see Australian Patent Application filed on 4 June 1998 entitled "Expression Modulating Sequences") 20 and negative regulatory sequences (see Australian Patent Application filed on 4 June 1998 entitled "Expression Modulating Sequences-II").

In work leading up to the present invention, the inventors identified yet a further regulatory mechanism involved in controlling plant physiological processes. The mechanism involves 25 modulating starch metabolism and/or cell shape and/or expansion and this in turn influences such phenomena as disease resistance, senescence, cell growth and the shape of cells, tissues and organs.

## SUMMARY OF THE INVENTION

Throughout this specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a 5 stated element or integer or group of elements or integers but not the exclusion of any other element or integer or group of elements or integers.

Sequence Identity Numbers (SEQ ID NOs.) for the nucleotide and amino acid sequences referred to in the specification are defined following the bibliography. A summary of SEQ ID NOs: is 10 given in Table 1.

One aspect of the present invention contemplates a method for controlling physiological processes in a plant said method comprising modulating starch metabolism in cells of said plant.

15 More particularly, the present invention is directed to a method of inducing a physiological response in a plant said method comprising enhancing or facilitating starch metabolism in cells of said plant after the initial development stage.

Another aspect of the present invention provides a method of inducing a physiological response 20 in a plant such as but not limited to inducing resistance to a plant pathogen, enhancing or delaying senescence, modifying cell growth or altering the shape of cells, tissues or organs, said method comprising modulating synthesis of an amylase or functional derivative thereof for a time and under conditions sufficient for starch metabolism to be facilitated or inhibited.

25 Still another aspect of the present invention relates to a transgenic plant or a genetically modified plant exhibiting one or more of the following properties:

- (i) a non-developmentally silenced amylase gene;
- (ii) an amylase gene capable of constitutive or inducible expression;
- 30 (iii) a mutation preventing silencing of an amylase gene;

- (iv) a nucleic acid molecule proximal to an amylase gene and which substantially prevents methylation of said amylase gene; and/or
- (v) decreased amylase gene expression.

5 Another aspect of the present invention contemplates a method for controlling physiological processes in a plant said method comprising modulating cell shape and/or expansion in said plant.

More particularly, the present invention is directed to a method of inducing a physiological response in a plant said method comprising enhancing or facilitating the manipulation of cell  
10 shape and/or expansion in said plant.

Still another aspect of the present invention provides a method of inducing a physiological response in a plant such as but not limited to inducing resistance to a plant pathogen, enhancing or delaying senescence, modifying cell growth or altering the shape of cells, tissues or organs,  
15 said method comprising modulating expression of the *Dem* gene.

Yet still another aspect of the present invention relates to a transgenic plant or a genetically modified plant exhibiting one or more of the following properties:

20 (i) a non-developmentally silenced *Dem* gene;  
(ii) a *Dem* gene capable of constitutive or inducible expression;  
(iii) a mutation preventing silencing of the *Dem* gene;  
(iv) a nucleic acid molecule proximal to the *Dem* gene and which substantially prevents methylation of said *Dem* gene or demethylates the *Dem* gene; and/or  
25 (v) decreased *Dem* gene expression.

Another aspect of the present invention contemplates a method for controlling physiological processes in a plant said method comprising modulating C metabolism in cells of said plant.

More particularly, the present invention is directed to a method of inducing a physiological response in a plant said method comprising enhancing or facilitating C metabolism in cells of said plant.

5 Still another aspect of the present invention provides a method of inducing a physiological response in a plant such as but not limited to inducing resistance to a plant pathogen, enhancing or delaying senescence, modifying cell growth or altering the shape of cells, tissues or organs, said method comprising modulating expression of a putative patatin gene or a functional derivative thereof.

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Yet still another aspect of the present invention relates to a transgenic plant or a genetically modified plant exhibiting one or more of the following properties:

- (i) a non-developmentally silenced putative patatin gene;
- 15 (ii) a putative patatin gene capable of constitutive or inducible expression;
- (iii) a mutation preventing silencing of a putative patatin gene;
- (iv) a nucleic acid molecule proximal to a putative patatin gene and which substantially prevents methylation of said putative patatin gene or demethylates said putative patatin gene; and/or
- 20 (v) decreased putative patatin gene expression.

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**TABLE 1**  
**SUMMARY OF SEQ ID NOs.**

<b>SEQ ID NO.</b>	<b>DESCRIPTION</b>
5	1 Nucleotide sequence of tomato $\alpha$ -amylase gene promoter
	2 Nucleotide sequence of potato $\alpha$ -amylase gene promoter
	3 Nucleotide sequence of genomic DNA upstream of <i>Dem</i> gene followed by <i>Dem</i> cDNA coding sequence
	4 Nucleotide sequence of putative <i>Dem</i> promoter
	5 Nucleotide sequence upstream of <i>Ds</i> insertion (ie. upstream of the <i>nos:BAR</i> gene) in a putative patatin gene in tomato
10	6 Nucleotide sequence downstream of <i>Ds</i> insertion (ie. downstream of the <i>nos:BAR</i> gene) in a putative patatin gene in tomato
	7 Nucleotide sequence of portion of putative tomato homologue of potato patatin gene
	8 Nucleotide sequence of portion of potato patatin gene

**BRIEF DESCRIPTION OF THE FIGURES**

**Figure 1** is a diagrammatic representation showing T-DNA regions of binary vectors carrying a *Ds* element (SLJ1561) of the transposable gene (SLJ10512)[5]. The *Ds* element carries a 5 *nos:BAR* gene and is inserted into a *nos:SPEC* excision marker. The transposon gene *sAc* is linked to a 2':*Gus* reporter gene.

**Figure 2** is a diagrammatic representation showing an experimental strategy for generating tomato lines carrying transposed *Ds* elements (5). F1 plants heterozygous for both the *Ds* and 10 *sAc* T-DNAs are test-crossed to produce TC<sub>1</sub> progeny. The TC<sub>1</sub> progeny are then screened for lines carrying a transposed *Ds* and a reactivated *nos:BAR* gene.

**Figure 3** is a representation of a sequence comparison between the potato  $\alpha$ -amylase promoter [SEQ ID NO:2] (14) and the tomato  $\alpha$ -amylase promoter [SEQ ID NO:1]. The location of the 15 UQ406 insertion is shown in bold.

**Figure 4** is a diagrammatic representation showing the chromosomal region of the tomato  $\alpha$ -amylase, *Dem* and  $\gamma$  genes. The  $\alpha$ -amylase and  $\gamma$  coding sequences are shown as shaded boxes and the *Dem* gene as an open box on the chromosome. The region of homology to the potato 20  $\alpha$ -amylase promoter and coding sequence are shown on the figure.

**Figure 5** is a photographic representation showing tissue and *in situ* distribution of *Dem* mRNA. a, Northern blot analysis of *Dem* expression in light-grown seedlings (LS), dark-grown seedlings (DS), shoot apices (SA), mature leaves (ML), young fruit (YF), roots (R), stem (S) and callus 25 (C). b-d, *in situ* hybridization with a *Dem* antisense probe. b, shoot apical meristem of a 4 week-old plant. c, dormant auxiliary meristem. d, root apex.

**Figure 6** is a photographic representation showing somatic tagging of the *Dem* locus. a, leaf showing the somatic tagging of the *Dem* locus. Light coloured sectors on the adaxial side of the 30 leaf represent independent insertions of *Ds* in *Dem*. The appearance of the abaxial side of the leaf is the same as wild-type. b, Scanning Electron Microscope (SEM) of a somatic sector

showing abnormal and wild-type epidermal cells. The SEM shows a wild-type sector in the lower right hand half of the figure, and a mutant sector in the upper left hand side. Note that the epidermal and hair cells are larger on the wild-type sector.

5 **Figure 7** is a representation showing that the *Dem* gene is required for palisade cell expansion in the leaf. Transverse sections of (a) variegated and (b) wild-type leaves. **p** and **s** indicate a palisade cell and spongy mesophyll cell layers, respectively. Light green parts are indicated by **lg**, and green parts by **g**. Light green sectors lacking palisade cells are mutated by *Ds* insertion in the *Dem* gene.

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**Figure 8** shows PCR on intact tissue of *dem* sectors. **M**, 1 kb ladder. **1-10**, unique *Ds* insertions in *Dem* detected by PCR. Intact leaf tissues (mutant somatic sectors) were used as template in the PCR. PCR with oligonucleotide primers facing out of *Ds* and in the *Dem* coding sequence amplified unique fragments from each mutant sector, thereby confirming that the sectors shown 15 in Figures 6 and 7 are indeed mutant *dem* sectors.

20 **Figure 9** is a diagrammatic representation showing an improved transposon tagging strategy using *Dem* as excision marker. The *sAc* and *Ds* parent lines are represented by the upper left and right boxes, respectively. Because the stabilised *sAc* is linked to the frameshift *dem* allele in one parent, somatic revertants occur at the frequency of about 1 out of 4 in the F1 progeny. Each somatic revertant represents an independent transposition event. Chr4, chromosome 4 of tomato.

25 **Figure 10** is a representation of the nucleotide sequence [SEQ ID NO:3] of genomic DNA from 651 bp upstream of the *Ds* insertion in UQ406 to the beginning of the *Dem* coding sequence, followed by the *Dem* cDNA sequence from the ATG start site at base pair 4097. The target sequences of UQ406 and *Dem* ATG are underlined. The *Dem* cDNA sequence is shown in italics and is underlined. The putative *Dem* promoter is 709 bases long beginning at nucleotide 3388 and ending just prior to the ATG, i.e. at position 4096 [SEQ ID NO:4].

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**Figure 11** is a photographic representation showing the dominant lesion mimic phenotype of UQ406. The leaf tissue on the left is wild-type, on the right is UQ406. Young and old leaves are shown in the upper and lower portions of the figure, respectively. No symptoms have been observed on young differentiating tissue of UQ406.

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**Figure 12** is a diagrammatic representation of the genetic derivation of plants containing independent somatic *dem* alleles. Somatic revertants were generated by crossing plants heterozygous for the *dem*<sup>+7</sup> mutant allele linked to transposase (sAc,GUS) and plants heterozygous for the *dem*<sup>Ds</sup> mutant allele. Revertant seedlings were selfed and GUS<sup>+</sup> individuals 10 were identified. From 150 somatic revertants, four independent lines were produced carrying hundreds of independent *dem* alleles.

**Figure 13** is a photographic representation showing a multicellular palisade mutant allele of the *Dem* locus. At the single-cell embryo stage, the plant possessing the multicellular palisade sector 15 shown carried a transposase gene and was heterozygous for a mutant frameshift allele and a wild-type allele of the *Dem* locus. During development, however, mutant *dem* sectors were produced due to the insertion of a *Ds* element into the wild-type allele. Wild-type palisade tissue is essentially composed of single long columnar cells. Some mutant sectors (due to *Ds* insertion) totally lack palisade cells (refer to the figure), whereas other mutant sectors have multicellular 20 palisade tissue composed of small, non-columnar cells.

**DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS**

In accordance with the present invention, transposon-mediated tagging of tomato plants was shown to result in the identification of mutants exhibiting altered physiological properties. In particular, the insertion of a transposon in close proximity to the  $\alpha$ -amylase gene resulted in continued or modified expression of the  $\alpha$ -amylase gene past the initial development stage of the plant. In wild-type plants, negative regulatory mechanisms which may include methylation result in the non-expression of the  $\alpha$ -amylase gene. In accordance with the present invention, modified expression of the  $\alpha$ -amylase gene, post or after initial developmental stage, results in physiological attributes such as altered senescence, altered resistance to pathogens, modification of the shape of plant cells, tissues and organs and altered cell growth characteristics. It is proposed, in accordance with the present invention, that the altered physiological phenotype is due to modified starch metabolism by the continued or modified expression of the  $\alpha$ -amylase gene. In particular, increased or modified expression of the  $\alpha$ -amylase gene or otherwise continued or altered expression of the  $\alpha$ -amylase gene post initial development results in cell death, i.e. cell apoptosis, but also induces or promotes resistance to pathogens.

Accordingly, one aspect of the present invention contemplates a method for controlling physiological processes in a plant said method comprising modulating starch metabolism in cells of said plant.

More particularly, the present invention is directed to a method of inducing a physiological response in a plant said method comprising inhibiting or facilitating starch metabolism in cells of said plant after the initial developmental stage.

25

The present invention is exemplified herein with respect to the effects of starch metabolism in tomato plants. This is done, however, with the understanding that the present invention extends to the manipulation of starch metabolism in any plant such as flowering plants, crop plants, ornamental plants, vegetable plants, native Australian plants as well as Australian and non-Australian trees, shrubs and bushes.

Physiological responses contemplated by the present invention include but are not limited to cell apoptosis, senescence, pathogen resistance, cell, tissue and organ shape and plant growth.

In a particularly preferred embodiment, starch metabolism is stimulated, promoted or otherwise 5 enhanced or inhibited by manipulating levels of an amylase and this in turn may lead to *inter alia* senescence or apoptosis as well as resistance to pathogens. Reference to "amylase" includes any amylase associated with starch metabolism including  $\alpha$ -amylase and  $\beta$ -amylase. This aspect of the present invention also includes mutant amylases. In addition, the manipulation of levels of amylase may be by modulating endogenous levels of a target plant's own amylase, or an 10 exogenous amylase gene or antisense, co-suppression or ribozyme construct may be introduced into a plant. The exogenous amylase gene may be from another species or variety of plant or from the same species or variety or from the same plant. The present invention extends to recombinant amylases and derivative amylases including fusion molecules, hybrid molecules and amylases with altered substrate specifications and/or altered regulation.

15

According to another aspect of the present invention there is provided a method of inducing a physiological response in a plant such as but not limited to inducing resistance to a plant pathogen, enhancing or delaying senescence, modifying cell growth or altering the shape of cells, tissues or organs, said method comprising modulating synthesis of an amylase or functional 20 derivative thereof for a time and under conditions sufficient for starch metabolism to be modified.

Preferably, the amylase is  $\alpha$ -amylase.

The manipulation of amylase levels may be by manipulating the promoter for the amylase gene, 25 inhibiting or promoting negative regulatory mechanisms such as described in an Australian Patent Application filed on 4 June 1998 entitled "Expression Modulating Sequences - II" or introducing anti-methylation sequences such as those described in an Australian Patent Application filed on 4 June 1998 entitled "Expression Modulating Sequences". Alternatively, an exogenous amylase gene may be introduced or an exogenous promoter designed to enhance expression of the 30 endogenous amylase gene.

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The present invention further extends to a transgenic plant or a genetically modified plant exhibiting one or more of the following characteristics:

- (i) a non-developmentally silenced amylase gene;
- 5 (ii) an amylase gene capable of constitutive or inducible expression;
- (iii) a mutation preventing silencing of an amylase gene;
- (iv) a nucleic acid molecule proximal to an amylase gene and which substantially prevents methylation of said amylase gene; and/or
- (v) decreased amylase gene expression.

10

The term "proximal" is used in its most general sense to include the position of the amylase gene near, close to or in the genetic vicinity of the nucleic acid molecule referred to in part (iv) above. More particularly, the term "proximal" is taken herein to mean that the amylase gene precedes, follows or is flanked by the nucleic acid molecule. Preferably, the amylase is within the nucleic acid molecule and, hence, is flanked by portions of the nucleic acid molecule. Generally, the amylase gene is flanked by up to about 100 kb either side of the nucleic acid molecule, more preferably up to about 10 kb, even more preferably to about 4 kb either side of the nucleic acid molecule and even more preferably up to about 10 bp to about 1 kb.

20 Accordingly, another aspect of the present invention is directed to an isolated nucleic acid molecule comprising a sequence of nucleotides which stabilises, increases or enhances expression of an amylase gene inserted into, flanked by, adjacent to or otherwise proximal to the said nucleic acid molecule.

25 In an alternative embodiment, the present invention contemplates an isolated nucleic acid molecule comprising a sequence of nucleotides which inhibits, decreases or otherwise reduces expression of an amylase gene inserted into, flanked by, adjacent to or otherwise proximal to the said nucleic acid molecule.

30

The term "expression" is conveniently determined in terms of desired phenotype. Accordingly, the expression of a nucleotide sequence may be determined by a measurable phenotypic change such as resistance to a plant pathogen, enhanced or delayed senescence, altered cell growth or altered cell, tissue or organ shape.

5

The nucleic acid molecule described above is referred to herein as an "expression modulating sequence" (EMS) since it functions to and is capable of modulating expression of an amylase gene or its derivatives. The term "modulating" includes increasing or stabilising expression of the amylase gene or decreasing or inhibiting the amylase gene. An EMS may be a co-suppression 10 molecule, ribozyme, antisense molecule, an anti-methylation sequence, a methylation-inducing sequence and/or a negative regulatory sequence, amongst other molecules.

Accordingly, another aspect of the present invention relates to an expression modulating sequence (EMS) comprising a sequence of nucleotides which increases, enhances or stabilizes 15 expression of an amylase gene inserted within, adjacent to or otherwise proximal with said EMS.

In an alternative embodiment, the present invention provides an expression modulating sequence (EMS) comprising a sequence of nucleotides which inhibits, decreases or otherwise reduces expression of an amylase gene inserted within, adjacent to or otherwise proximal with said EMS.

20

Another aspect of the present invention contemplates a genetic construct comprising an EMS as herein defined and means to facilitate insertion of a nucleotide sequence within, adjacent to or otherwise proximal with said EMS wherein said nucleotide sequence encodes an amylase or functional derivative thereof.

25

The term "genetic construct" is used in its broadest sense to include any recombinant nucleic acid molecule and includes a vector, binary vector, recombinant virus and gene construct.

The means to facilitate insertion of a nucleotide sequence include but are not limited to one or 30 more restriction endonuclease sites, homologous recombination, transposon insertion, random insertion and primer and site-directed insertion mutagenesis. Preferably, however, the means is

one or more restriction endonuclease sites. In the case of the latter, the nucleic acid molecule is cleaved and another nucleotide sequence ligated into the cleaved nucleic acid molecule.

Preferably, the amylase gene sequence is operably linked to a promoter in the genetic construct.

5

According to this embodiment, there is provided a genetic construct comprising an EMS as herein defined and means to facilitate insertion of a nucleotide sequence within, adjacent to or otherwise proximal with said EMS and operably linked to a promoter wherein said nucleotide sequence encodes an amylase or functional derivative thereof.

10

Conveniently, the genetic construct may be a transposable element such as but not limited to a modified form of *Ds*. A modified form of *Ds* includes a *Ds* molecule comprising an EMS and a nucleotide sequence such as but not limited to a reporter gene and a gene encoding an amylase.

15

Another aspect of the present invention contemplates a method of increasing or stabilising expression of a nucleotide sequence encoding an amylase or otherwise preventing or reducing silencing of a nucleotide sequence encoding an amylase in a plant cell said method comprising introducing into said plant or plant cells said nucleotide sequence encoding an amylase flanked by, adjacent to or otherwise proximal with an EMS.

20

In an alternative embodiment, the present invention provides a method of inhibiting, decreasing or otherwise reducing expression of a nucleotide sequence encoding an amylase in a plant cell said method comprising introducing into said plant or plant cells said nucleotide sequence encoding an amylase flanked by, adjacent to or otherwise proximal with an EMS.

25

Yet another aspect of the present invention provides a transgenic plant carrying a nucleotide sequence encoding an amylase flanked by, adjacent to or otherwise proximal with an EMS.

30

Still a further aspect of the present invention provides nucleic acid molecules encoding apoptotic peptides, polypeptides or proteins or nucleic acid molecules which themselves confer apoptosis. One example of an apoptotic nucleic acid molecule is a molecule capable of inducing or

enhancing amylase synthesis. Other molecules are readily identified, for example, by a differential assay. In this example, nucleic acid sequences (e.g. DNA, cDNA, mRNA) are isolated from wild type plants and mutant plants which exhibit enhanced or modified amylase gene expression. The differential assay seeks to identify DNA or mRNA molecules in the mutant

5 plant or wild type plant which are absent in the respective wild type plant or mutant plant. Such nucleic acid molecules are deemed putative apoptosis-inducing or apoptosis-inhibiting genetic sequences. These molecules may have utility in regulating beneficial physiological processes in plants.

10 The present invention is further directed to the putative *Dem* promoter and its further derivatives. This is approximately 709 bases in length extending upstream from the ATG start site. The nucleotide positions of putative *Dem* promoter are nucleotide 3388 to 4096 (Figure 10).

Another aspect of the present invention contemplates a method for controlling physiological  
15 processes in a plant said method comprising modulating cell shape and/or expansion in said plant.

More particularly, the present invention is directed to a method of inducing a physiological response in a plant said method comprising enhancing or facilitating the manipulation of cell shape and/or expansion in said plant.

20 This aspect of the present invention is based on the detection of a *Ds* insertion in the *Dem* gene in plants. The resulting mutation results in genetically-modified palisade tissue. Mutant lines exhibiting altered cell shape or expansion are selected and, in turn, further lines exhibiting such beneficial characteristics as increased levels of photosynthetic activity are obtainable.

25 Accordingly, another aspect of the present invention provides a method of inducing a physiological response in a plant such as but not limited to inducing resistance to a plant pathogen, enhancing or delaying senescence, modifying cell growth or altering the shape of cells, tissues or organs, said method comprising modulating expression of the *Dem* gene.

Still yet another aspect of the present invention relates to a transgenic plant or a genetically modified plant exhibiting one or more of the following properties:

- (i) a non-developmentally silenced *Dem* gene;
- 5 (ii) a *Dem* gene capable of constitutive or inducible expression;
- (iii) a mutation preventing silencing of the *Dem* gene;
- (iv) a nucleic acid molecule proximal to the *Dem* gene and which substantially prevents methylation of said *Dem* gene or demethylates the *Dem* gene; and/or
- (v) decreased *Dem* gene expression.

10

Yet another aspect of the present invention is directed to a mutation in or altered expression of a putative patatin gene in tomato or other plants. The patatin gene is referred to herein as "putative" as it exhibits homology to the potato patatin gene.

15 Accordingly, another aspect of the present invention contemplates a method for controlling physiological processes in a plant said method comprising modulating C metabolism in cells of said plant.

More particularly, the present invention is directed to a method of inducing a physiological 20 response in a plant said method comprising enhancing or facilitating C metabolism in cells of said plant.

Another aspect of the present invention provides a method of inducing a physiological response in a plant such as but not limited to inducing resistance to a plant pathogen, enhancing or 25 delaying senescence, modifying cell growth or altering the shape of cells, tissues or organs, said method comprising modulating expression of a putative patatin gene or a functional derivative thereof.

Still yet another aspect of the present invention relates to a transgenic plant or a genetically modified plant exhibiting one or more of the following properties:

- (i) a non-developmentally silenced putative patatin gene;
- 5 (ii) a putative patatin gene capable of constitutive or inducible expression;
- (iii) a mutation preventing silencing of a putative patatin gene;
- (iv) a nucleic acid molecule proximal to a putative patatin gene and which substantially prevents methylation of said putative patatin gene or demethylates said putative patatin gene; and/or
- 10 (v) decreased putative patatin gene expression.

The present invention is further described by the following non-limiting Examples.

**EXAMPLE 1*****Ds* Transposon tagging of an  $\alpha$ -amylase gene affecting plant development**

The inventors have previously developed a two component *Ds/sAc* transposon system in 5 transgenic tomato for tagging and cloning important genes from plants (5, 12). The components of the system are shown in Figure 1 and comprise: i) a non-autonomous genetically-engineered *Ds* element (e.g. SLJ1561), and ii) an unlinked transposase gene *sAc* (SLJ10512), required for transposition of the *Ds* element. To activate transposition, the two components are combined by crossing transformants for each component. A plant selectable 10 marker gene, e.g. *nos:BAR*, is inserted into the *Ds* element to enable selection for reinsertion of the elements following excision from the T-DNA (Figure 1). Surprisingly, the marker gene is irreversibly inactivated when the *Ds* line is crossed to a transformant expressing the transposase gene (5). Silencing occurred when the *Ds* element remained in the T-DNA, and also occurred in the great majority of cases when the *Ds* element transposed to a new location 15 in the tomato genome. None of the other marker genes in the T-DNA is silenced. The silenced marker gene has been shown to be stably inherited, even after the transposase gene segregates away from the *Ds* element in subsequent generations.

The experimental strategy for generating tomato lines carrying transposed *Ds* elements from 20 T-DNA 1561E is shown in Figure 2. One line, called UQ406, carries a single transposed *Ds* element (without the transposase gene which has segregated away) and is characterised by showing a disease mimic or premature senescence phenotype on mature leaves. UQ406 also possesses an active *nos:BAR* gene indicating that the insertion caused two phenotypes; namely premature senescence and reactivation of the *nos:BAR* gene inside the *Ds* element.

25

GenomeWalker (13) is used to clone the tomato DNA sequences flanking the *Ds* element in UQ406. The DNA flanking the *Ds* element in line UQ406 is cloned and sequenced, and a search of the PROSITE database reveals that the *Ds* has inserted into the promoter region of an  $\alpha$ -amylase gene. The promoter shows strong homology to an  $\alpha$ -amylase promoter of potato 30 (14; Figure 3) and the coding sequence of the gene has strong homology with one of 3 reported potato  $\alpha$ -amylase cDNAs (15). Surprisingly, DNA sequence analysis also shows that the *Ds*

insertion in UQ406 is located only about 3 kb upstream from the ATG of the *Dem* (Defective embryo and meristems) gene which has been cloned by tagging with *Ds*. In fact, only about 700 bp of DNA separates the putative  $\alpha$ -amylase STOP codon and the *Dem* ATG codon (Figure 4). The *Dem* gene is required for correct patterning in all of the major sites of differentiation, 5 namely in the embryo, meristems, and organ primordia (Figure 5). The inventors have shown by somatically tagging *Dem* with *Ds*, that the gene is involved in cell expansion during plant differentiation (Figures 6, 7 and 8). The close proximity of the  $\alpha$ -amylase and *Dem* genes indicates that the  $\alpha$ -amylase gene may also be involved in cell expansion during plant differentiation. The sequence flanking the active *nos:BAR* genes are referred to herein as 10 "Expression Modulating Sequences" or "EMSs".

## EXAMPLE 2

### An improved transposon tagging strategy for transgenic tomato

15 The inventors have used the transposon tagging system described in Example 1 (also see Figure 2) to tag and clone three important genes involved in shoot morphogenesis: the *DCL* gene, required for chloroplast development and palisade cell morphogenesis (12); the *Dem* gene, required for cotyledon development and shoot meristem function; and the  $\alpha$ -amylase gene, described in Example 1 above.

20 Stable *Ds* insertion mutants of *Dem* germinate but fail to develop any further. However, variegated seedlings appear at first to be mutant, but the transposase gene activates transposition of the *Ds* and reversion of the *Dem* locus to wild-type, thereby restoring function to the shoot meristem. While the transposon tagging system described in Figure 2 has been successful in 25 tagging genes and chromosomal regions alleviating transgene silencing, it does have two associated inefficiencies. First, transposition cannot be selected in the shoot meristem of  $F_1$  plants heterozygous for *Ds* and *sAc*. As a consequence, many  $TC_1$  progeny derived from test-crossing these  $F_1$  plants still have the *Ds* located in the T-DNA. The other limitation of the system is that sibling  $TC_1$  progeny derived from a single  $F_1$  plant often carry the same clonal 30 transposition and reinsertion event. The extent of clonal events amongst sibling  $TC_1$  progeny can only be monitored by time consuming and expensive Southern hybridization.

These two inefficiencies in the transposon tagging strategy are overcome in accordance with the present invention by using the *Dem* gene as an excision marker. The new system enables selection for transposition in the shoot apical meristem and visual identification of plants carrying 5 independent transposition events. Transposition is initiated by crossing a *Ds* line with a *sAc* line (Figure 9). The *Ds* line is heterozygous for a *Ds* insertion in the *Dem* gene and the *sAc* line is heterozygous for a stable frameshift mutation in the *Dem* gene (Figure 9). The frameshift allele is derived from a *Ds* excision event from the *Dem* locus. Both the *Ds* and *sAc* lines are wild-type due to the recessive nature of the *Ds* insertion and frameshift alleles. PCR tests on intact leaf 10 tissue have been developed for the rapid identification of these *Ds* and *sAc* parental lines. The *F*<sub>1</sub> progeny derived from crossing the *Ds* and *sAc* lines segregate at the expected ratio of 3 wild-types to 1 mutant. Because the stabilised *sAc* is linked to the frameshift *dem* allele almost all of the *F*<sub>1</sub> mutants also inherit the transposase gene (*sAc*) and can undergo somatic reversion. These revertant individuals have abnormal cotyledons, but *Ds* excision from the *Dem* gene restores 15 function to the shoot apical meristem. Each somatic revertant represents an independent transposition event from the *Dem* locus. A non-destructive test for *nos:BAR* expression is used involving application of PPT (the selective agent for expression of *BAR* gene) to a small area of a leaf. Somatic revertants resistant to PPT are grown though to seed and the *F*<sub>2</sub> progeny are screened again for PPT resistance. Lines carrying transposed *Ds* elements are selected for more 20 detailed molecular analysis. Independent *Ds* insertions in the vicinity of *Dem* and the  $\alpha$ -amylase gene are identified by PCR.

### EXAMPLE 3

#### Modification of plant cell, tissues and organ shapes and plant growth by genetic manipulation of $\alpha$ -amylase

25

The DNA from 651 bp of the upstream of the UQ406 insertion down to the end of the *Dem* coding sequence has been sequenced (Figure 10). The close proximity of the  $\alpha$ -amylase gene to the *Dem* cell expansion gene indicates that these genes may play a key role in cell expansion 30 and differentiation. Several heterozygous insertion mutants are identified in the  $\alpha$ -amylase coding sequence and these are selfed to produce plants homozygous for the *Ds* insertion in the  $\alpha$ -

amylase coding sequence. If these have a similar or more or less severe phenotype to the plants homozygous for the stable *Dem* insertion mutant, then this will indicate that indeed this cloned  $\alpha$ -amylase gene plays a key role in cell expansion, and, therefore, the shape and growth of plants. Several heterozygous insertion mutants have been identified in the  $\gamma$  coding sequence 5 downstream of the *Dem* coding sequence (Figure 4) and these are selfed to produce plants homozygous for the *Ds* insertion in the  $\gamma$  coding sequence. If these have a similar or more or less severe phenotype to the plants homozygous for the stable *Dem* insertion mutant, then this will indicate that the  $\gamma$  gene also has a role in cell expansion and the shape and growth of plants.

10 A tomato chromosomal region spanning these genes is cloned into an *Agrobacterium* binary vector (16) to produce plasmid pUQ113, and this plasmid is introduced into *Arabidopsis* by method of (17) to modify the cell shape and growth of this other plant species. A T-DNA insertion mutant in the *Dem* gene is identified in *Arabidopsis* and this mutant is also transformed with pUQ113 to modify the cell shape and growth of *Arabidopsis*.

15

Recombinant combinations of  $\alpha$ -amylase and *Dem* genes are transformed into a range of plant species to modify the cell shape and growth of the species.

#### EXAMPLE 4

20     **Genetic engineering of disease resistance and senescence based on modification  
                  of expression of  $\alpha$ -amylase**

*Ds* insertion mutant UQ406 is characterized by a lesion mimic phenotype. The mutant phenotype is evident in mature leaves (Figure 11), but not in young leaves or any other tissue. No pathogens 25 are found in leaf tissue displaying this phenotype. The dominant nature of the UQ406 phenotype and the location of the *Ds* in the  $\alpha$ -amylase promoter suggest that over-, under or constitutive expression of the gene may be responsible for activating a disease resistance response and/or senescence in mature leaves. These data and the very close proximity of the  $\alpha$ -amylase and *Dem* genes are also consistent with co-ordinate regulation of these genes in differentiating tissue.

30 Induction of disease resistance and plant senescence, to produce desirable outcomes in crops and

plant products, may, therefore, be able to be controlled by modification of  $\alpha$ -amylase expression.

An early event in the disease response of a challenged plant is a major respiratory burst, often referred to as an oxidative burst due to an increase in oxygen consumption. This burst of oxygen 5 consumption is due to the production of hydrogen peroxide ( $H_2O_2$ ) linked to a surge in hexose monophosphate shunt activity (19). This activity results from the activation of a membrane-bound NADPH oxidase system which catalyses the single electron reduction of oxygen to form superoxide ( $HO_2/O_2^-$ ), using NADPH as the reductant (19). Spontaneous dismutation of  $HO_2/O_2^-$  then yields  $H_2O_2$ . Consumption of glucose *via* the hexose monophosphate shunt 10 (alternatively known as the cytosolic oxidative pentose phosphate pathway) regenerates the NADPH consumed by the NADPH oxidase system. It is, therefore, entirely conceivable that an  $\alpha$ -amylase is responsible for supplying sugars required by the pentose phosphate pathway, and perhaps for the primary activation of the signal transduction pathway that leads to disease resistance in plants.

15

Following the oxidative burst, disease resistance is manifested in localised plant cell death called the hypersensitive response (HR), in the vicinity of the pathogen. The HR may then induce a form of long-lasting, broad spectrum, systemic and commercially important resistance known as 20 systemic acquired resistance (SAR). The compounds, salicylic acid, jasmonic acid and their methyl derivatives as well as a group of proteins known as pathogenesis related (PR) proteins are used as indicators of the induction of SAR (18).

Increased levels of sugars have been related to heightened resistance especially to biotrophic 25 pathogens (20). When invertase (the enzyme responsible for the breakdown of sucrose to glucose and fructose) is overexpressed in transgenic tobacco, systemic acquired resistance is induced (21).

The  $\alpha$ -amylase coding sequence is inserted behind an inducible promoter and transformed into plants to confer a inducible disease resistance in plants. Similarly, the  $\alpha$ -amylase coding 30 sequence is inserted behind an inducible promoter and transformed into plants to confer inducible senescence in plants for the production of desirable products or traits.

When a disease resistance response is invoked in one part of a plant, a general and systemic acquired enhancement in disease resistance is conferred on all tissues of such a plant (18). Tomato line UQ406 is tested for enhanced resistance to a wide range of pathogens to test this hypothesis.

5

#### EXAMPLE 5

##### Modification of the photosynthetic architecture of plants

A genotype has been produced for the somatic tagging of the *Dem* gene, thereby demonstrating 10 the involvement of the *Dem* gene in cell expansion. The genetic derivation of somatically-tagged *Dem* is shown in Figure 12.

Besides palisade-less sectors, another type of mutant sector has been identified in somatically-tagged *Dem* plants. The new phenotypic class is characterized by multicellular palisade tissue. 15 In the wild-type tomato, the palisade tissue is composed of a single long columnar palisade cell. In the new mutant sectors, which look wild-type to the naked eye, the long columnar cell is replaced by several smaller cells packed on top of one another. This is shown in Figure 13. Each mutant sector arises from an independent insertion of *Ds* in the *Dem* gene. The different classes of mutant sectors apparently result from different classes of mutations in the *Dem* gene.

20

Somatically-tagged *Dem* plants are crossed to a stable null mutant of *Dem* and progeny are screened to identify stable mutant lines with genetically-modified palisade tissue. Lines exhibiting beneficial characteristics, such as increased levels of photosynthetic activity, can then be selected. Lines resulting from other *Dem* alleles and exhibiting other beneficial modifications, for example 25 altered developmental architecture such as modified cell, tissue or organ growth rate, shape or form, may also be identified.

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### EXAMPLE 6

#### Ds transposon tagging of a putative patatin gene

Other lines carrying transposed *Ds* elements have also been selected.

5

DNA sequences flanking the active *nos:BAR* in a line designated UQ12 have similarly been cloned and sequenced. The flanking DNA appears to correspond to an intron in a homologous potato patatin gene. Patatin is the major protein in the potato tuber and has many potentially-  
important characteristics. For example, it possesses antioxidant activity; it has esterase activity  
10 and is potentially a phospholipase or lipid acyl hydrolase (hydrolyzing phospholipids, liberating free fatty acids); it is induced during disease resistance; and it inhibits insect larval growth.

The sequence upstream of the *Ds* insertion (i.e. upstream of the *nos:BAR* gene) is as follows:

15	AATCAAAGAG GAATTNAATT CCNCAAAATT TCATCCATAG ATTTTGNNGTC	50
	TCTGAAAATT AAAGTGACTT TGTAATCTGA AACCTAGAGT CCTCAACCAT	100
	ATCATTGACC ATTAAGCCAT ACCCTTAAAT GTAGGGAATT TGAAGTTTTA	150
	AAAACCACAC TTTGTTATT ATTGGGCCAA ATACTCGATA ATCTTTACAT	200
	TATTGAAAAT CAACATTCAA AAGGAACGAA CCTTCAATCA CACCATCAAT	250
20	GTCAACTTTC TTTTATTTG GATAATCTAA GTTTTTAAAT TGCACTAAAAA	300
	TNAAATAAAA CCCTAAACTT CTTCTAGGTT GAGACTTAGT AAATATGAAT	350
	TATATAAAGA ATTCACTGACA AATGAGACAT AAGAATAGTG CCAGCAAATT	400
	ACTTTTTGGA TATCTTATCT GTGATATCGG AATTTTAACT ACCATAAAATT	450
	TATGAATGAA ATATCACTTA TCTATTAGAG AGGATTTAAT CTCCCTTATA	500
25	ATGACATTGA TAAAAGCAAG NACAAGTGCT CTTTATTCT TAATTACAAA	550
	TCCTTAAATA GATAAAAGCT ACGAATAACA TAATATCCTT AAATAGATAAA	600
	AAGCTACGAA TAACATAATA GTATATTACT CCNAATTATT TTGATTTATT	650
	TTAAATGACT CCACTAATCC TGATGTGGTC TAGG [SEQ ID NO:5]	684

30

The tomato sequence immediately downstream of the *Ds* insertion (i.e. downstream of the *nos:BAR* gene), is as follows:

5	GGTCTAGGCC CTGGGTCTAG GAAACAAAAT AACTTATTTG ACTCCTAAAC AATAGCAACA TACAAACCCAC TGATATTGTA CAAGTAAAAT TCAATAAAAT TCTAGCTCTC TCAAACACTT TTAAAATTGT TATTTCTGTT TTGTCCTGTT CATATTATGA CCTACACAAAC AACAAACAACA ACGAATTTAG TGAAACTCTA CAAAGTGGAG CCTGAAGTCG AGAGTTACG CGGGCCTTAT CACTATCTT TCGAGATAAA AAAATTATTT TTAAAAGATC ATCGACTTAA ACAAAACAAA	50 100 150 200 250 300
10	CAATAATTAA AAAAATATGA ATTAATAGCA AAGCAGTGTG GACCATATAT ACAAAAATCT ATAACAACAA CAAGGTGCAG AGCATTATTC CAACTAAGAT CGAAGTTGTG ATACTGTCAT AATAAAAATG ACACATATTT TGACAAACATA AAAAATAAAAT AACCATAAAA TATATCATAG AAAAATGAAT ATATTAGAAC AGCTCACTCC AATATTAAAA GAGAGAAAAA AAATATTTTC CCACCACAAT	350 400 450 500 550
15	GCCATAATCC TTGAGCTTAG CTATTTATAA GTAAAAAAAAA TGTTTTCTTG GATAAAATAGA AAAAGAAATA ATAATTAAAC ATAACCAATC ACTTCACAAAA TAAGAGTGTAA TT [SEQ ID NO:6]	600 650 662

The level of homology between the potato and our tomato sequence is as follows:

20 Tomato: 307 ATTTATTTTAGAAAAAATTATCTAAATACACATCTTATTTACCATATACTCTAAAAAT 248

||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||

Potato: 1914 AATTATATTTAGGAAAAATTACATAAATACACAACCTTAATATATTATTTCTCTAAATT 1973

247 TCC 245 [SEQ ID NO:7]

25

1974 TCC 1976 [SEQ ID NO:8]

**EXAMPLE 7****Tagging of additional genes involved in carbon metabolism**

This *Ds* line also exhibits a disease mimic phenotype, indicating that the patatin gene may be 5 involved in disease resistance and/or may act as an anti-oxidant in plant cells.

Selecting for transposition of a methylated *Ds* from the *Dem* locus and for expression of the *nos:BAR* gene (i.e.: demethylation of the *Ds*) efficiently identifies *Ds* insertions into genes, as opposed to so-called "junk DNA". The sequences adjacent to five of these *Ds* insertions have 10 been cloned and sequenced, and in all of the cases the *Ds* insertion is in the vicinity of a known gene.

The five lines carrying active *nos:BAR* genes associated with genes are:

- *Ds* insertion in UQ406 - associated with the promoter of an  $\alpha$ -amylase gene (Example 15 1, above);
- *Ds* insertion in UQ12 - associated with a putative patatin gene (Example 6, above);
- *Ds* insertion in UQ11 - associated with the Right Border of the *Agrobacterium* T-DNA in 1516E (refer to Figure 2). This was the T-DNA carrying the *Ds* that was initially transformed into tomato. In other words, the *Ds* transposed from the *Dem* locus back 20 into the T-DNA;
- *Ds* insertion in UQ14 - associated with or closely linked to a putative sucrose synthase gene (Example 8, below); and
- *Ds* insertion in UQ13 - associated with or closely linked to a putative UDP-glucose-pyrophosphorylase gene, a gene potentially involved in starch biosynthesis.

25

In four of these instances, the *Ds* is associated with a gene related to carbon (C) metabolism ( $\alpha$ -amylase, patatin, sucrose synthase and UDP-glucose-pyrophosphorylase). The lines designated UQ12 and UQ14 are also characterised by a disease mimic phenotype, implying that a patatin gene and a sucrose synthase gene (and probably other C metabolism genes) are involved in 30 disease resistance.

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**EXAMPLE 8**  
**Modifications of carbon metabolism**

As stated above, in four of the five lines carrying active demethylated *nos:BAR* genes, the *Ds* has 5 inserted into or near sequences homologous with carbon metabolism gene. These results indicated that many C metabolism genes have *cis*-acting sequences which prevent methylation and concomitant gene silencing. Demethylation sequences are inserted next to recombinant C metabolism genes to enhance their expression and modify C metabolism in beneficial ways; such as up-regulation of the sucrose phosphate synthase gene in sugar cane, to yield higher 10 concentrations of sugar in beneficially-modified plants.

**EXAMPLE 9**  
**Cloning of downstream genes associated with plant cell apoptosis**  
**caused by *Ds* insertion**

15 A cDNA library is made from tomato leaf tissue showing the disease mimic (apoptosis) phenotype caused by *Ds* insertion. This library is screened differentially with two probes, one being cDNA from normal tissue and the other being cDNA made from leaf tissue showing the disease mimic phenotype caused by *Ds* insertion. This procedures identifies genes specifically- 20 induced during plant cell death. These apoptosis-associated genes are then sequenced, and compared with other genes present in the DNA databases. The proteins encoded by these genes are expressed *in vitro* and tested for their ability to kill plant cells.

Those skilled in the art will appreciate that the invention described herein is susceptible to 25 variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or features.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: THE UNIVERSITY OF QUEENSLAND

(ii) TITLE OF INVENTION: A METHOD FOR MODULATING PLANT PHYSIOLOGICAL PROCESSES AND GENETIC SEQUENCES USEFUL FOR SAME-II

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(B) COMPUTER: IBM PC compatible  
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- 30 -

## (2) INFORMATION FOR SEQ ID NO:1:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1217 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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CATTATCACT	GAGCCTTATG	ATTATGTTT	ACGAGCTTAT	AATATCACTG	ATGGTGATTC	180
AGTATTGTGA	TTATGTCCTT	CGTTGATTAT	TCTGTTTCA	ACAAGTCGTG	TAATTTGCTG	240
TTTGTGACAG	TACGATAGAT	CGACTCAACC	TTCTGAGGTA	TTAGTTGAAG	TTCATGTAAA	300
TTAGCTTGTT	TTATCATAGT	AGCATTGAT	TATTGATGCT	CTGTAGCTAA	TGATAAGCCA	360
TTGGAGGGAA	GCAAGCTTTC	TAAATGAATC	TACGAATGGA	TGATAAAAGTT	CATGAATATT	420
TTTGTACTT	CTGCAGTCAG	ATCATGAGTT	ATTGAGTCTA	TTGTTTTTT	AAGCCTGTT	480
CAGATGATCC	ATCATCAGTA	ACAACATACA	CGGTGTAGTC	CCAAATCCAT	CATATGCACC	540
TTCTTTCTT	CAATTGGTC	TTGTTTTTT	TTTTCATGA	TGTCATTGAA	TTATTCAAGA	600
AGTCACTTCG	AGCATAATGA	TTTTCAAAA	TCCACCTTG	TTCAAGCACT	ACCACGTCTT	660
TTCATCTAGC	CCACAACCGT	GGTGGAGGAT	CTAGAATT	CATGAAAGGA	TTCAAAATTT	720
ACAAACATAT	ATATACACTA	TACACTATGA	ATCCACTAAT	ACTAGATGGT	GCACCTGTGC	780
CCCCACTCAT	GTAAGGCCT	ATTCTCAATT	TTTTATTTTC	CACAACCTAA	ATACAGACCG	840
CACAACCTCC	GTGTCTTGTG	TGCTCGTCGC	TCAGCATGCA	AGTCGAGAAA	AGAAAGACCA	900
AAACAATGAA	AACTTACGA	AAAATCAAAA	AGTTGAAGGA	CTTTAACGTC	GAGATCTCTC	960
GTAGAAAACC	TCTTTGTAA	GGTTGCATAC	AATACTTTT	TTTCAGACTT	TACTTATGGT	1020
ATTATACTGA	ATATGTTATT	GCTGTTATAG	TAGTTGAGTG	ACGTTGAGG	GAATTCTAG	1080
TCCGTTAAC	TTGTACTCAG	TGTGTCTACT	TTTCAAAAAA	GTCAGTTTT	CAGTCTCTAA	1140
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## (2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1114 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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TATCACCGAA	CCTTATGATT	ATGTGTACGA	GCTTATAATA	TTACTGATGG	TGATTCACTA	180
TTATGATTAT	GTCCTCCATT	AATTATTCTG	TTTCATACAA	GTCTGTAAAT	TTGCTGTTG	240
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GTCGAGTTCT	ATAGTAAACA	ACCCCTATAT	CTTTTTCAA	GCATGTTAAG	ATTGCGAAC	960
CACTGAAATT	TCCAGGTCGT	TAATCTTGT	CCCAGTGTGT	GTACTTTAA	AAAAAAAAGT	1020
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TACTAGACTT	CGGAGTCAAC	ACAACACAAC	AACA			1114

## (2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 6263 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

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AACTTCATCA	TCATACAGTA	TGGTTTGAT	ATGCTCTCC	ATTATCACTG	AGCCTATGA	180
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GCATTTGATT	ATTGATGCTC	TGTAGCTAAT	GATAAGCCAT	TGGAGGGAAG	CAAGCTTCT	420
AAATGAATCT	ACGAATGGAT	GATAAAAGTTC	ATGAATATTT	TTGTTACTTC	TGCAGTCAGA	480
TCATGAGTTA	TTGAGTCTAT	TGTTTTTTA	AGCCTGTTTC	AGATGATCCA	TCATCAGTAA	540
CAACATACAC	GGTGTAGTCC	CAAATCCATC	ATATGCACCT	TCTTTCTTC	AATTTGGTCT	600
TGTTTTTTT	TTTCATGAT	GTCATTGAAT	TATTCAAGAA	GTCACTTCGA	GCATAATGAT	660
TTTTCAAAAT	CCACCTTGT	TCAAGCACTA	CCACGTCTT	TCATCTAGCC	CACAACCGTG	720
GTGGAGGATC	TAGAATTTC	ATGAAAGGAT	TCAAAATTAA	CAAACATATA	TATACACTAT	780
ACACTATGAA	TCCACTAATA	CTAGATGGT	CACCTGTGCC	CCCACCATG	TGAAAGCCTA	840
TTCTCAATT	TTTATTTCC	ACAACCTAAA	TACAGACCGC	ACAACCTCCG	TGTCTGTGT	900
GCTCGTCGCT	CAGCATGCAA	GTCGAGAAAA	GAAAGACCAA	AACAATGAAA	ACTTTACGAA	960
AAATCAAAAA	GTTGAAGGAC	TTAACGTG	AGATCTCTG	TAGAAAACCT	CTTTGTAAG	1020
GTTGCATACA	ATACTTTT	TTCAGACTTT	ACTTATGGTA	TTATACTGAA	TATGTTATTG	1080
CTGTTATAGT	AGTTGAGTGA	CGTTGAGGG	AATTCTAGT	CCGTTAATCT	TGTACTCAGT	1140
GTGTCTACTT	TTCAAAAAAG	TCAGTTTTC	AGTCTCTAAA	ACACATTAA	ATAAGAGTTT	1200
CTTTGCCCAT	CTTTGTTCC	TCATCCTAGG	CTTGGAGTCA	ACACAACACA	ACAACAATGA	1260
ATTTCCATT	TTCTGTTCT	TTACTTCTCT	CTTTATCTCT	TCCTATGTT	GCCTCTCGA	1320
CGGTGTTATT	TCAGGTATCC	ATCTCAAAG	AACCTTATT	TTCTCTAAC	TTTCCTATG	1380
TATATGTATC	TCTATGTTA	TGTAGTACTT	GCTCAAGTAT	ATAAAGAAAA	GTTAGTTCT	1440
CTAGAACCTT	TGAATTCAATT	TGTTAGGGT	TCAATTGGGA	TTCGAGTAAT	AAGCAAGGCG	1500
GATGGTACAA	CTCTCTCATC	AACTTAGTTC	CGGACTTGGC	TAAAGCTGGA	GTTACTCATG	1560
TTGGTTGCC	ACCATCATCT	CACTCCGTT	CTCCTCAAGG	TAATTTCGG	AGTGATTGTG	1620
ACCTAGTAAT	CCAATGAAGT	CAAATAACC	ACGGAAGATT	AGAGTCTAAA	TTTAATGAA	1680
AATAGTCAG	ACAAGTTAAT	GACCAACTTA	TATATTAGTT	CAATCCATAA	AATTGATGT	1740
AGTAGTTACA	AAATGGAATT	GCTTGAAGGC	TTATGCCATG	TTTATGCCA	GGTTATATGC	1800
CAGGAAGGTT	GTATGACTAG	GATGCTTCCA	AGTTGGAAA	TCAGCAACAA	CTGAAAACCTC	1860
TTATTAAGGC	TTAACATGA	CCACGGGATC	AAATCGGTTG	CTGATATAGT	GATAAATCAT	1920
AGAACTGCTG	ATAACAAAGA	TAGCAGGGGA	ATATACAGCA	TCTTGAAGG	AGGAACATCT	1980
GATGACCGGC	TTGATTGGGG	TCCATCTTC	ATTTGCAGGA	ACGACACACA	ATATTCTGAT	2040
GGCACGGGGA	ATCCAGACAC	GGGTTGGAC	TTTGAACCTG	CACCTGATAT	CGATCATCTT	2100
AATACGAGAG	TGCAGAAAGA	GTTATCAGAC	TGGATGAAC	GGCTGAAATC	TGAAATTGGA	2160

TTTGATGGTT	GGCGTTTCGA	TTTTGTTAGG	GGATATGCAC	CTTGCATTAC	CAAAATTTAT	2220
ATGGGAAACA	CGTCCCCGGA	TTTTGCTGTT	GGTGAATTGT	GGAACTCTCT	TGCTTATGGC	2280
CAGGACGGGA	AACCGGAATA	TAACCAGGAC	AATCATAGAA	ATGAGCTAGT	TGGTTGGGTA	2340
AAAAATGCGG	GGCGGGCTGT	AACAGCTTT	GATTTACAA	CAAAGGGAAT	TCTTCAAGCT	2400
GCAGTTCAAG	AAGAGTTATG	GAGATTGAAG	GATCCAATG	GAAAACCTCC	TGGGATGATC	2460
GGTGTGTTGC	CTCGAAAAGC	TGTGACTTT	ATCGATAATC	ATGATACTGG	ATCGACACAA	2520
AATATGTGGC	CTTTCCCTTC	AGACAAAGTT	ATGCAAGGAT	ATGCATACAT	TCTTACTCAT	2580
CCAGGAATCC	CATCCGTGGT	AAAAAAAATA	AATAAATTCT	TTCTACATAT	CTCATTGTT	2640
TCTATTTAC	AAGAAATTAA	TATTCTTTTC	CAGGGGATTT	GAGAAACTCG	GCCTGTGGGA	2700
GTTTGCTCAC	ATTGCCAGTC	TCGTAATCCA	AAACAAACA	CTCAAACCTCT	GAGTGTGCAC	2760
ATCTAGACAC	CTCAACTCGT	TTTCACCGT	GTAAATTGAA	CACTTCAACT	TACAAAATGA	2820
TCGTGTAGCA	CCTCCAAAAA	TTATGTGTCA	CAATTAGCCA	CGTGCAGAGAT	ACACGAAAAT	2880
GAGTTGGAGT	AGTTAGTTGC	CAAATAAAAC	CAAGCTGAGG	TGTCTAAATG	TGCACNCTCA	2940
AAGTNGGATG	TTTACTTGGC	AGCTGAGGCC	GAGGCCATGT	TTGANTGTTA	TGCTTATAGG	3000
ATATGACACA	TTTGTGTTCCG	ATTAGCTGAG	GANTTGATTA	AATCCTNGTT	TTNGTTNGCA	3060
GTTTNATNAC	CATTNCTTTG	ATNGGGGCTN	CNAGGATGGA	ATTNCAGCAC	TAANCTCTAT	3120
TAGGAAAAGG	AATAGGATTT	GTGCANCAAG	CAATGTGCAA	ATAATGGCTC	CTGATTCTGA	3180
ATCTTATAT	ANCAATGGAT	CATCACAAAA	TCATTGTCAA	GATTGGACCA	AAACTTGATC	3240
TTGGAAATCT	TATTCCACCT	AATTATGAGG	TGGCAACTTC	TGGACAAGAC	TATGCTGTAT	3300
GGGAGCAAAA	GGCATAATCA	TATTGTACCA	CACTAAAAGG	GACCATGGCC	ACAATGGTTC	3360
TCATTAGTGT	TAATGTTATA	TGATTGAAAA	TGTAATTAT	ATTGACATAA	TGAAGGCCAA	3420
AAATTCAAGA	AATTATAAAC	AATTCAATAG	TCCTTGCTCA	ATTCAACAATT	ACATTATGAC	3480
TTCTCTATTG	CAAACATAGTT	TGGGTCCACA	TTATTGTCTC	CTAAAATTCT	ACAACATTTC	3540
TTAAGGAAAC	TTAATTAGTT	ACAGTGAACA	TATGTGAAA	TTACCCCTTA	TCCCCTTACA	3600
ATTGATTAA	AAAATATTTC	CCCTATCCCT	TTGGTAGTTG	GTTAGAGTTA	TAAGTAACGT	3660
AGAGATTAGT	TATAAGAGAA	TTTATGTATT	ATTATGCAGA	TGTTTAGTTA	TATCGATTCT	3720
AGTTATTAT	ATGTTGATTA	TTTCACCTTC	AATAATGCAT	ATAAAGATGG	TAAATGATTG	3780
GATTGATCGA	ATTCGAATGA	GGTTGAATAT	GAACTAATCT	TCAAATTAA	TATAAATTCT	3840
TTTTGTCAAC	ATCTATAGCC	AAACGGCTCC	AAAACAATAA	ATAATTACAA	TTTATTGTAG	3900
TATTTTATT	AAAATGGAT	NTTCCTCATC	CCACTTGTAC	CAGTTGAAAC	CCTAATAATA	3960
AGCCAATCCA	ACCGTCAAAA	TTACAAATT	TGAAAATTGC	GCTCCTCACA	GTTCTCCCT	4020
ATTCAGATT	GATTCAATTCT	CTTCATTCTT	TGTTTCACA	TTTTACCTCT	AAATCAACTC	4080
GAGTCCCTTT	GTTCAAATGG	GTGCTAATCA	CAGCCGTGAA	GATCTGGAGC	TTTCTGATTC	4140
CGAGTCTGAA	TCCGAATATG	GGTCCGAGTC	TCGAACAAGG	GAGGAAGAGG	AAGACGAAGA	4200

TAACTACTCA GATGCTAAAA CGACGCCGTC TTCCACTGAT CGGAAACAGA GCAAAACCCC	4260
GTCTTCTTG GATGATGTTG AAGCAAAGCT GAAAGCTTTA AAGCTTAAGT ATGGTACTCC	4320
TCATGCTAAA ACCCCCCACAG CGAAAAACGC TGTTAAACTT TACCTTCATG TTGGTGGGAA	4380
CACTGCGAAT TCCAAATGGG TAGTTCTGA TAAGGTGACA GCTTATTCTGT TTGTTAAATC	4440
GGGTAGTGAG GATGGATCGG ATGATGATGA AAATGAAGAA ACTGAGGAGA ATGCTTGGTG	4500
GGTTTGAAA ATTGGGTCGA AGGTTGGGC TAAGATTGAT GAGAATTGTC AGCTCAAGGC	4560
ATTTAAGGAG CAGAAAAGGG TGGATTGTG GCGAATGGG GTTGGGCTG TGAGATTCTT	4620
TGGGGAGGAA GAGTATAAGG CGTTCATGAT CTTATATCAG AGCTGTTGT TTGAGAATAC	4680
TTATGGGTTT GAGGCAAATG ATGAGAATAG AGTTAAGGTG TATGGTAAAG ACTTTATGGG	4740
GTGGGCAAAT CCAGAAGCTG CGGATGATTC AATGTGGGAG GATGCTGGGG ATAGCTTCGC	4800
GAAGAGCCCT GCGTCTGAAA AGAAGACACC TTTGAGGGTT AACCATGATT TGAGGGAGGA	4860
GTGGGAGGAG GCAGCTAAAG GAGGAGCTAT TCAGAGCTTG GCATTAGGTG CGTTGGATAA	4920
TAGTTTCTT ATAAGTGATT CTGGAATTCA GGTTGTGAGG AACTATACTC ATGGAATAAG	4980
TGGAAAAGGT GTTTGTGTCA ATTTGATAA GGAAAGGTCT GCTGTACCTA ATTCCACTCC	5040
AAGGAAAGCT CTACTTCTAA GAGCTGAGAC TAATATGCTT CTCATGAGTC CAGTGAUTGA	5100
TAGAAAGCCT CACTCTCGGG GATTACATCA GTTGTATATC GAGACTGGGA AGGGATTTAG	5160
CGAGTGGAAAG TTTGAGAAAG ATGGAACTGA TATCACGATG AGGGATATCA CTAATGATAG	5220
CAAAGGAGCT CAGATGGATC CTTGGGGTC TACTTTCTTA GGGCTAGATG ATAACAGATT	5280
GTGTAGGTGG GATATGCGTG ATCGGCATGG GATGGTCCAG AATCTAGTTG ATGAAAGTAC	5340
TCCTGTGCTG AATTGGACTC AAGGACATCA ATTTTGAGG GGAACTAAC TTCAGTGCTT	5400
TGCTACTACT GGTGATGGAT CAATTGTTGT TGGTTCACTT GATGGCAAGA TTAGATTGTA	5460
CTCAAGCAGT TCCATGAGAC AGGCTAAAC TGCTTTCCA GGCCTTGGTT CTCCTATCAC	5520
TCATGTGGAT GTTACCTATG ATGGGAAGTG GATATTGGGG ACAACTGATA CTTACTTGAT	5580
ATTGATATGC ACCTTGTAA TCGACAAGAA TGGAACTAAC AAGACTGGTT TTGCTGGTCG	5640
CATGGGAAAT AAGATTCCG CTCCAAGATT GTTAAAGCTA AACCTCTCG ATTACATAT	5700
GGCTGGAGCT AACAAAGTTCC GCAGTGCTCA ATTTTCATGG GTCACCGAGA ATGGGAAGCA	5760
AGAGCGCCAC CTCGTTGCTA CTGTTGGAA GTTGTGAGT ATCTGGAATT TTCAACAGGT	5820
GAAGGATGGT TCTCATGAGT GTTACCAAGAA TCAGGTTGGG TTGAAGAGCT GCTATTGTTA	5880
CAAGATAGTC CTAAGAGACG ACTCTATTGT AGAAAGTCGT TTCATGCATG ACAAGTACGC	5940
TGTTTCTGAC TCACCTGAAG CACCACTGGC GGTAGCAACC CCCATGAAAG TCAGCTCATT	6000
CAGCATCTCT AGCAGGCAGT TACAAATTG AACAAATCATT CTGTTCATAT ACGCAACTTA	6060
TTAGATTAT CTGTAGCAGA ATTAGTGCTC CTCACACTAA GTAGCTTGAA AAACTGCACA	6120
TCTGCAAATC ATTTCCAGTT CAATGTATTA CTACTTTAGT TTAAAAACCT TAAAAGGCAG	6180
TCTTCCAAAT TCTAGGTATC CTCACCTGAC ATTATTATTG TTGTAATAGC TAATTGTTGC	6240

TTGCTCTAAA TCCCCGTTCA ATG 6263

## (2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 708 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

AAATGTAATT TATATTGACA TAATGAAGGC CAAAATTCA AGAAATTATA AACAAATTCAA	60
TAGTCCTTGC TCAATTCAACA ATTACATTAT GACTTCTCTA TTGCAAACATA GTTGGGTCC	120
ACATTATTGT CTCCCTAAAT TTTACAACAT TTCTTAAGGG AACTTAATTAA GTTACAGTGA	180
ACATATGTTG AAATTACCCCT TTATCCCCT ACAATTGATT TAATAAATAT TTCCCCTATC	240
CCTTTGGTAG TTGGTTAGAG TTATAAGTAA CGTAGAGATT AGTTATAAGA GAATTATGTT	300
ATTATTATGC AGATGTTAG TTATATCGAT TTTAGTTATT TATATGTTGA TTATTCACC	360
TTCAATAATG CATATAAAGA TGGTAAATGA TTGGATTGAT CGAATTCGAA TGAGTTTGAA	420
TATGAACTAA TCTTCAAATT TAATATAAAT TTTTTTGTC AACATCTATA GCCAAACGGC	480
TCCAAAACAA TAAATAATTAC ACATTTATTG TAGTATTAA TTTAAAATGG GATTTCTCA	540
TCCCACCTGT ACCAGTTGAA ACCCTAATAA TAAGCCAATC CAACCGTCAA AATTACAAAT	600
TTTGAAAATT GCGCTCCTCA CAGTTCTCCC CTATTCAGAT TTGATTTCATT CTCTTCATT	660
TTTGTTTTCA CATTTCACCT CTAAATCAAC TCGAGTCCCT TTGTTCAA	708

## (2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 684 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

AATCAAAGAG GAATTNAATT CCNCAAAATT TCATCCATAG ATTTTGNGTC	50
TCTGAAAATT AAAGTGACTT TGTAATCTGA AACCTAGAGT CCTCAACCATT	100
ATCATTGACC ATTAAGCCAT ACCCTTAAAT GTAGGGAATT TGAAGTTTTA	150
AAAACCACAC TTTGTTATTT ATTGGCCCAA ATACTCGATA ATCTTTACAT	200
TATTGAAAAT CAACATTCAA AAGGAACGAA CCTTCAATCA CACCATCAAT	250
GTCAAACTTTC TTTTATTTG GATAATCTAA GTTTTAAAT TGCAGTAAAAA	300

TNAAATAAAA CCCTAAACTT CTTCTAGGTT GAGACTTAGT AAATATGAAT	350
TATATAAAGA ATTCATGACA AATGAGACAT AAGAATAGTG CCAGCAAATT	400
ACTTTTTGGA TATCTTATCT GTGATATCGG AATTTTAACt ACCATAAATT	450
TATGAATGAA ATATCACTTA TCTATTAGAG AGGATTTAAT CTCCCTTATA	500
ATGACATTGA TAAAAGCAAG NACAAGTGCT CTTTATTCT TAATTACAAA	550
TCCTTAAATA GATAAAAGCT ACGAATAACA TAATATCCTT AAATAGATAA	600
AAGCTACGAA TAACATAATA GTATATTACT CCNAATTATT TTGATTTATT	650
TAAAATGACT CCACTAATCC TGATGTGGTC TAGG	684

## (2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 662 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

GGTCTAGGCC CTGGGTCTAG GAAACAAAAT AACTTATTG ACTCCTAAC	50
AATAGCAACA TACAAACCAC TGATATTGTA CAAGTAAAAT TCAATAAAAT	100
TCTAGCTCTC TCAAACACTT TTAAAATTGT TATTCTGTT TTGTCTGTGT	150
CATATTATGA CCTACACAAAC AACAAACAACA ACGAATTAG TGAAACTCTA	200
CAAAGTGGAG CCTGAAGTCG AGAGTTACG CGGGCCTTAT CACTATCTTT	250
TCGAGATAAA AAAATTATTT TTAAAAGATC ATCGACTTAA ACAAAACAAA	300
CAATAATTAA AAAATATGA ATTAATAGCA AAGCAGTGTG GACCATATAT	350
ACAAAAATCT ATAACAACAA CAAGGTGCAG AGCATTATTC CAACTAAGAT	400
CGAAGTTGTG ATACTGTCAT AATAAAAATG ACACATATTT TGACAACATA	450
AAAAATAAAT AACCATAAAA TATATCATAG AAAATGAAT ATATTAGAAC	500
AGCTCACTCC AATATTAAAA GAGAGAAAAA AAATATTTC CCACCACAAAT	550
GCCATAATCC TTGAGCTTAG CTATTATCAA GTAAAAAAA TGTTTCTTG	600
GATAAAATAGA AAAAGAAATA ATAATTAAAC ATAACCAATC ACTTCACAAA	650
TAAGAGTGTAA TT	662

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(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 63 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

ATTTATTTTT AGGAAAAATT ATCTAAATAC ACATCTTATT TTACCATATA CTCTAAAAAT	60
TCC	63

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 63 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

AATTATATTT AGGAAAAATT ACATAAATAC ACAACTTAAT ATATTATATT CTCTAAAATT	60
TCC	63

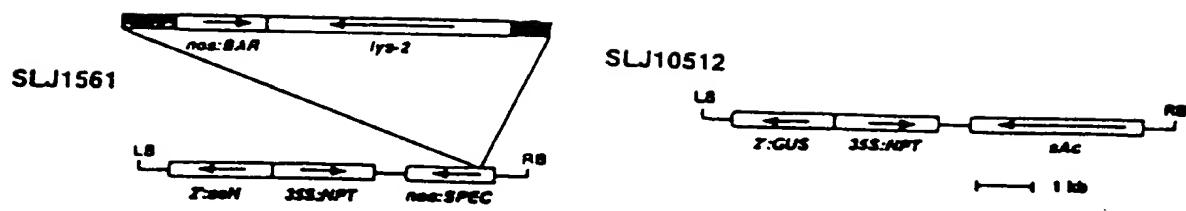
DATED this 25th day of September 1998

**THE UNIVERSITY OF QUEENSLAND**

By DAVIES COLLISON CAVE

Patent Attorneys for the Applicant

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**FIGURE 1**

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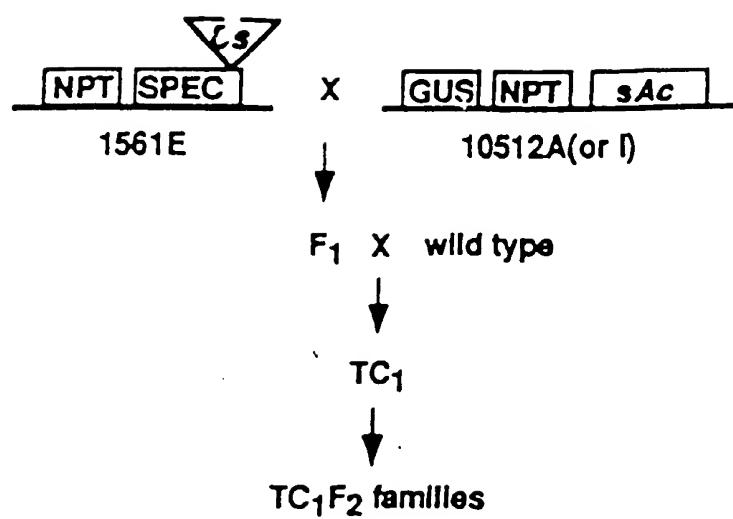


FIGURE 2

FIGURE 3 (i)

981	TTTGAAATTATGTATATATCTGTAGCATTAGAAACTATAAGAGTTGTTA	1030	Potato
40	TTTGAAATTATGTATTTATCTATAGCATTAGAAACTATAAGAGTTGTTA	89	Tomato
1031	GCTTCACCTGTCTTATTGTTGTGCTAAAGCAACT...TCATCATACAGT	1077	
90	GCTTCACCTGGCTACTGTTGTGCTAAAGCAACTTCATCATACAGT	139	
1078	ATGGTTTTATATGCTCTTCATTATCACCGAACCTTATGATTATG.TGT	1126	
140	ATGGTTTGATATGCTCTTCATTATCACTGAGCCTTATGATTATGTTT	189	
1127	ACGAGCTTATAATATTACTGATGGTATTCACTGAGCCTTATGATTATGTCCTC	1176	
190	ACGAGCTTATAATATCACTGATGGTATTCACTGAGCCTTATGATTATGTCCTT	239	
1177	CATTAATTATTCTGTTCATACAAGTCGTGTAATT'GCTGTTGTGATTG	1226	
240	CGTTGATTATTCTGTTCATACAAGTCGTGTAATTGCTGTTGTGACAG	289	
1227	TACGATAAAATTGATTCAACCTTCTGCGGTGTTGGTTGAAGTTCAAGTAAA	1276	
290	TACGATAGATCGACTCAACCTTCTGAGGTATTAGTTGAAGTTCATGTAATG	339	
1277	TTAGCTTTATTATCATAGTAGCATTGATTATTGATGCTCTGTAGCTAA	1326	
340	TTAGCTTTGTTATCATAGTAGCATTGATTATTGATGCTCTGTAGCTAA	389	
1327	TGATAAGCCATTGAAGGGAAAGCAGAAATGGTAAAGCTTCTAAAATGAAT	1376	
390	TGATAAGCCATTGGAGGGAAAGC.....AAGCTTCT.AAATGAAT	428	
1377	CTACGAATGGATGATAAAAGTTAATGAATATTGTTGATACTCTGCAATCA	1426	
429	CTACGAATGGATGATAAAAGTTCATGAATATTGTTGATACTCTGCAAGTC	478	
1427	GATTATGAGTTACTGAGCTACTG.TTTTTAAGCCTGTTTCAGATGATC	1475	
479	GATCATGAGTTATTGAGTCTATTGTTTTAAGCCTGTTTCAGATGATC	528	
1476	GATCATCAACAACACATATTCACTGAGTAGACATGATCGATCACTTC	1525	
529	CATCATCAGTAACAACACATACACGGTGTAGT..CCCAAATCCATCA.....	571	
1526	TAATTTGATTATGCACCCCTTTCTCAATTGGTC..GTCTTCTTT	1573	
572	.....TATGCACCTCTTTCTCAATTGGTCTGTTTTTT	610	
1574	TTTCATGATGTCAGTAATTATTCTCTGGTCGTCCCCACCATTAGGAA	1623	
611	TTTCATGATGTCATTGAATT.....ATTCAAGAA	640	
1624	GTC <b>ACTTCGAG</b> CATAATG...TGAAAACATCCACATT.TTCAA.....	1663	
641	GTC <b>ACTTCGAG</b> CATAATGATTTCAAAATCCACCTTGTCAAGCACTA	690	

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FIGURE 3 (ii)

1664 ..... ATCCAGC ..... AGAATTTC 1679  
 |||||  
 691 CCACGTCTTTCATCTAGCCCACAACCGTGGTGGAGGATCTAGAATTTC 740  
 |||||  
 1680 ATCAAACGGGGTTCAACATTAC... TACATGTATACTCTGAAGTCTG 1726  
 |||||  
 741 ATGAAA.. GGATTCAAAATTACAAACATATATACACTATACACTATG 788  
 |||||  
 1727 AATCCACTAATTCTAGATGGTGCATCTGTGCCCAACTTGTGAAAGCT 1776  
 |||||  
 789 AATCCACTAATACTAGATGGTGCACCTGTGCCCACTCATGTGAAAGCC 838  
 |||||  
 1777 TATTCTCAATTTTATTTCCAACAACCTGAATTGACGACACACAACTC 1826  
 |||||  
 839 TATTCTCAATTTTATTTCC.ACAACTAAATACAGACCGACAACTC 887  
 |||||  
 1827 CCGTGTCTTGT..... ACGGTCAAGCATCTGAGTGGAGAACTCAA... 1865  
 |||||  
 888 CCGTGTCTTGTGTGC CGTCGCTCAGCATGCAAGTCGAGAAAAGAAAGAC 937  
 |||||  
 1866 ..... TTAAGTGAACCTTAACG 1881  
 |||||  
 938 CAAAACAATGAAAACATTACGAAAATCAAAAGTTGAAGGACTTTAACG 987  
 |||||  
 1882 TCGAGTTCTATAGTAAACAAACCCCT..... ATATCTT 1913  
 |||||  
 988 TCGAGATCTCTCGTAGAAAACCTTTGTAAAGTTGCATACAATACTTT 1037  
 |||||  
 1914 TTTTCAAGCATGTTAAGATTGCGAACACACTGA..... 1946  
 |||||  
 1038 TTTTCAG.ACCTTACTTATGGTATTATACTGAATATGTTATTGCTGTTA 1086  
 |||||  
 1947 ..... AATTCCAGGTGTTAACCTTGACT 1972  
 |||||  
 1087 TAGTAGTTGAGTGACGTTGAGGGAAATTCTAGTCCGTTAACCTTGACT 1136  
 |||||  
 1973 CAGTGTGTACTTTAAAAAAAGTCAGTTTTAGTCTCTAAACA 2022  
 |||||  
 1137 CAGTGTGTCTACTTT... CAAAAAAAGTCAGTTTCAGTCTCTAAACA 1183  
 |||||  
 2023 CATTAAAT.AGAGTTATTTG.CCATCTTTGTTCCCTCATACTAGACTT 2070  
 |||||  
 1184 CATTAAATAAGAGTTCTTGCCCATCTTGTTCCCTCATCCTAGGCTT 1233  
 |||||  
 2071 CGGAGTCACACACACACAAACA 2094  
 |||||  
 1234 .GGAGTCACACACACACAAACA 1256

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FIGURE 4

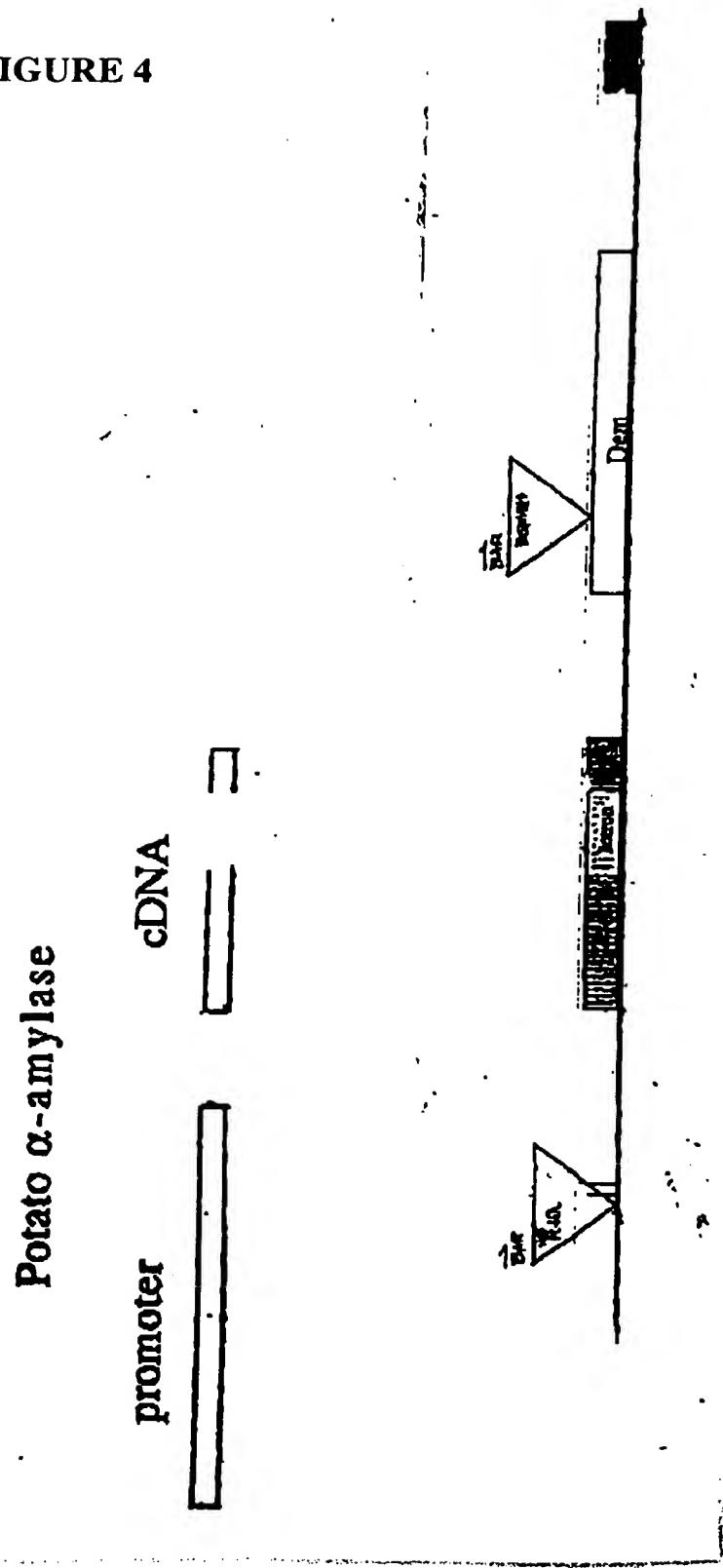
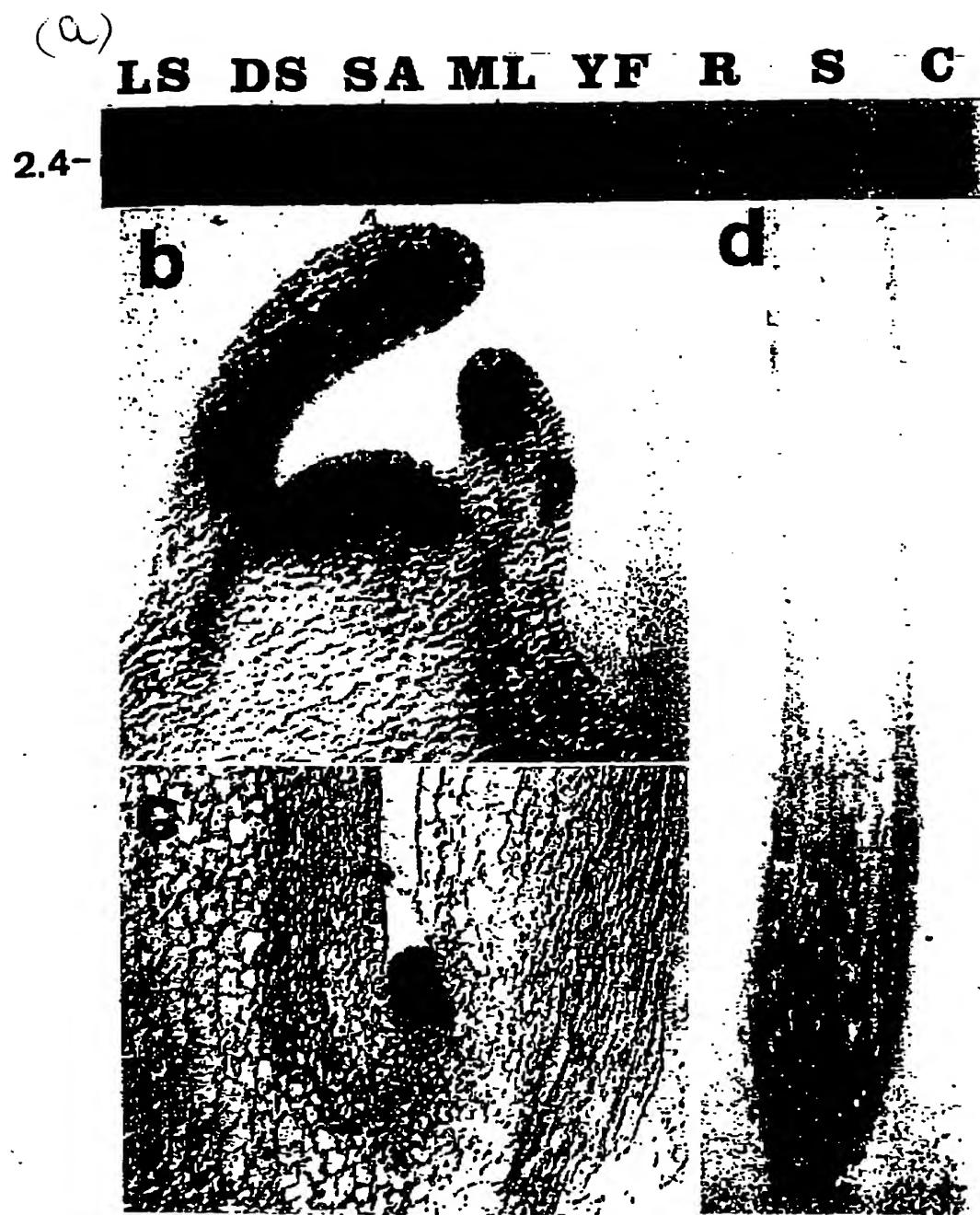


FIGURE 5



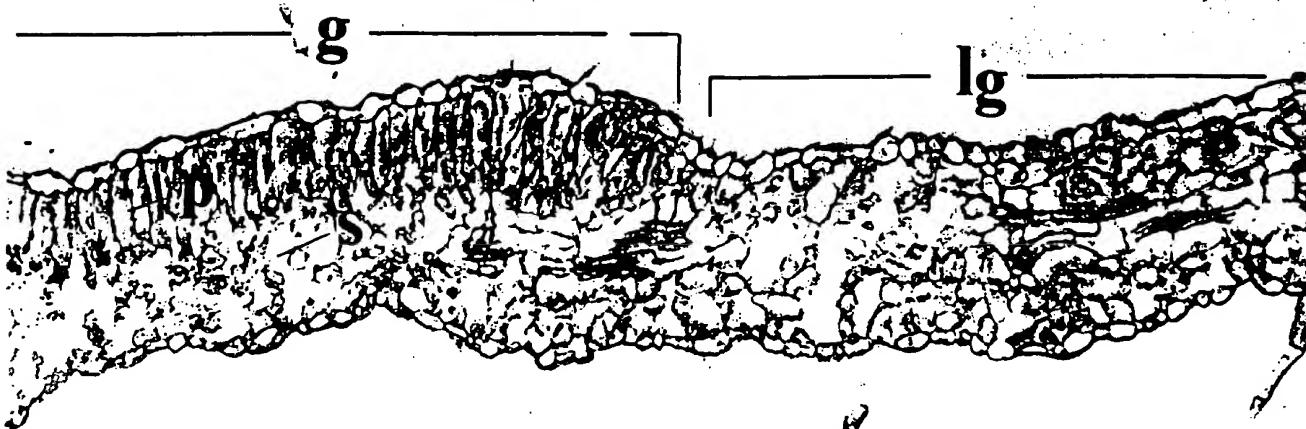
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**FIGURE 6**

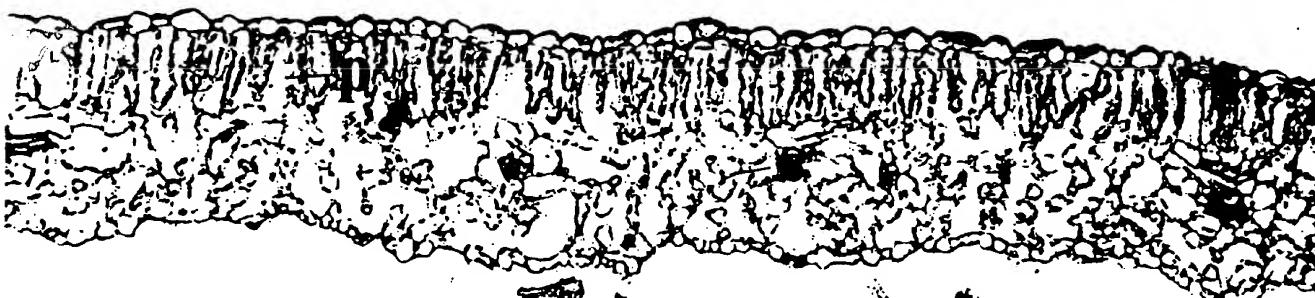


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**a**

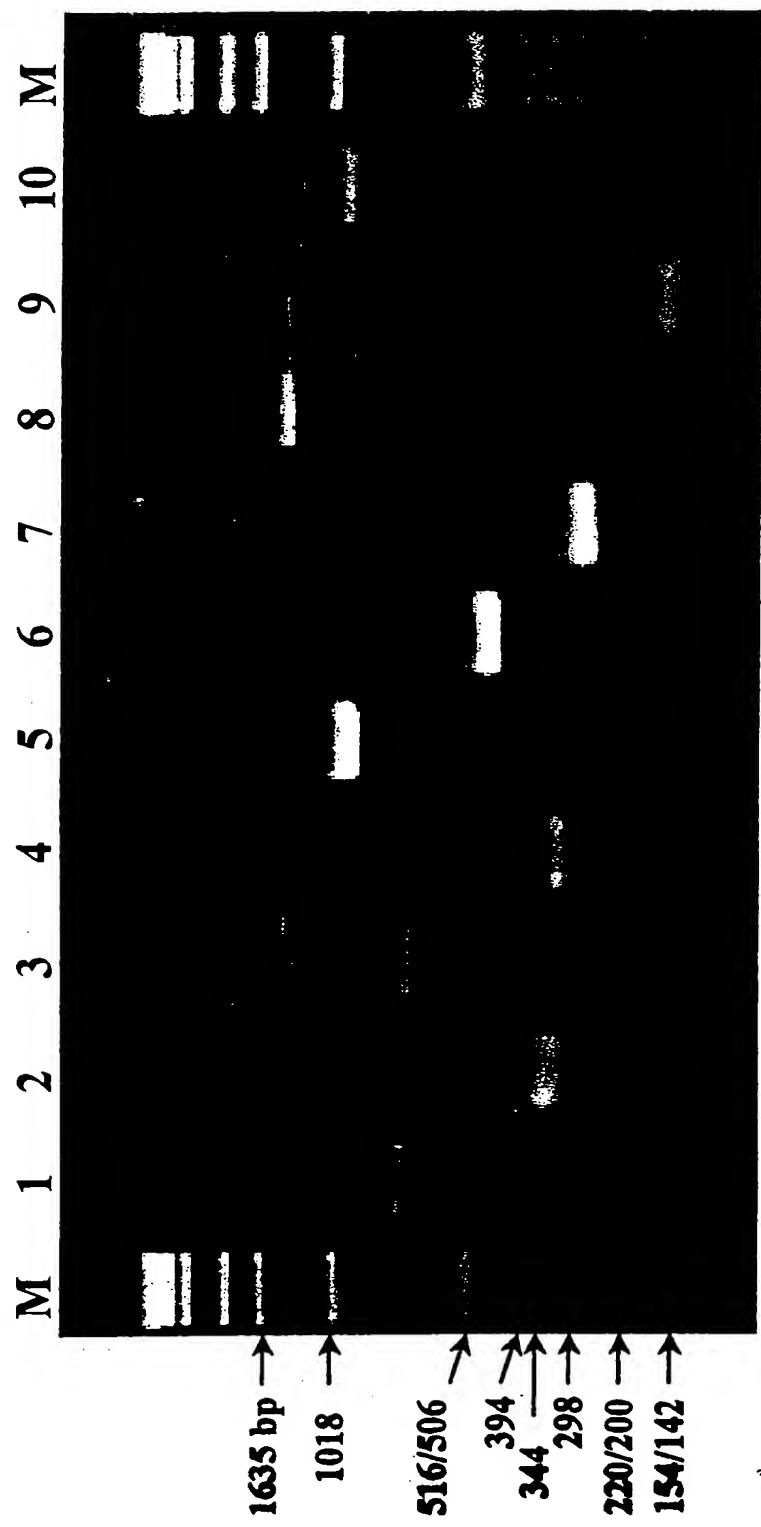
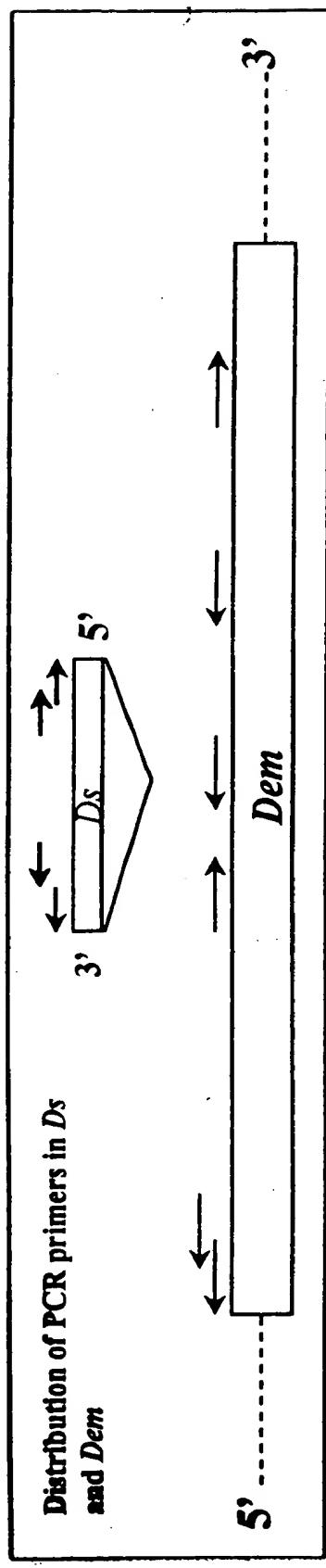


**b**



**FIGURE 7**

FIGURE 8



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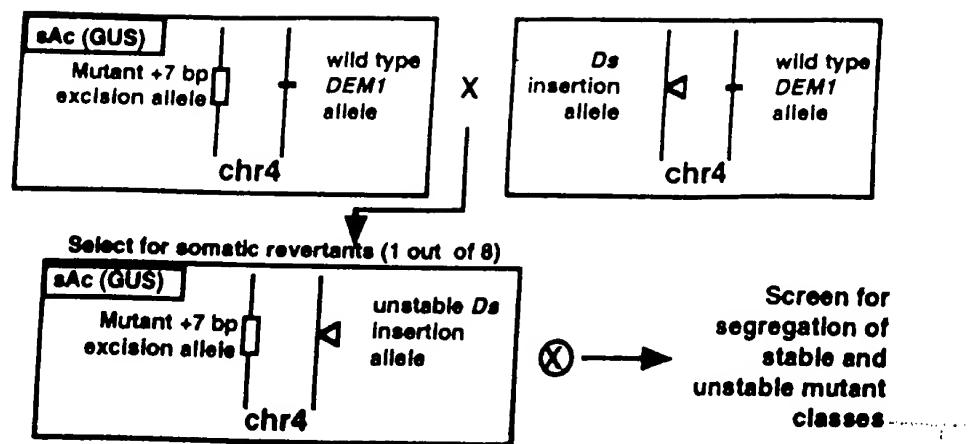


FIGURE 9

FIGURE 10 (i)

1 CGACGGCCCG GGCTGGTAAA TGCGGAAGCT TGTTACAGAT TTGAAATTAA  
 51 TGTATTTATC TATAGCATTA GAAACTATAA GAGTTGTTAG CTTCACTTGG  
 101 CTTACTGTTG TGCTCAAAGC AACTTCATCA TCATACAGTA TGGTTTTGAT  
 151 ATGCTCTTCC ATTATCACTG AGCCTTATGA TTATGTTTA CGAGCTTATA  
 201 ATATCACTGA TGGTGATTCA GTATTGTGAT TATGCTCTC GTTGATTATT  
 251 CTGTTTCATA CAAGTCGTGT AATTTGCTGT TTGTGACAGT ACGATAGATC  
 301 GACTCAACCT TCTGAGGTAT TAGTTGAAGT TCATGTAAT TAGCTTGT  
 351 TATCATAGTA GCATTGATT ATTGATGCTC TGTAGCTAAT GATAAGCCAT  
 401 TGGAGGGAAAG CAAGCTTTCT AAATGAATCT ACGAATGGAT GATAAAAGTTC  
 451 ATGAATAATT TTGTTACTTC TGCAGTCAGA TCATGAGTAA TTGAGTCTAT  
 501 TGTTTTTTA AGCCTGTTTC AGATGATCCA TCATCAGTAA CAACATACAC  
 551 GGTGTAGTCC CAAATCCATC ATATGCACCT TCTTTCTTC AATTGGTCT  
 601 TGTTTTTTT TTTTCATGAT GTCATTGAAT TATTCAAGAA GTCACCTTCGA uQ406  
 651 GCATAATGAT TTTCAAAAT CCACCTTTGT TCAAGCACTA CCACGTCTTT insertion  
 701 TCATCTAGCC CACAACCGTC GTGGAGGATC TAGAATTTC ATGAAAGGAT  
 751 TCAAAATTAA CAAACATATA TATACACTAT ACACATATGAA TCCACTAATA  
 801 CTAGATGGTG CACCTGTGCC CCCACTCATG TGAAAGCCTA TTCTCAATT  
 851 TTTATTTCAC ACAACTTAA TACAGACCGC ACAACTCCCG TGTCTTGT  
 901 GCTCGTCGCT CAGCATGCAA GTCGAGAAAA GAAAGACCAA AACAATGAAA  
 951 ACTTTACGAA AAATCAAAAA GTTGAAGGAC TTTAACGTG AGATCTCTCG  
 1001 TAGAAAAACCT CTTTGTAAAG GTTGCATACA ATACTTTTT TTCAAGACTTT  
 1051 ACTTATGGTA TTATACTGAA TATGTTATTG CTGTTATAGT AGTGTGAGTGA  
 1101 CGTTTGAGGG AATTTCAGT CCGTTAATCT TGTACTCAGT GTGTCTACTT  
 1151 TTCAAAAAAG TCAGTTTTC AGTCTCTAAA ACACATTAA ATAAGAGTTT  
 1201 CTTTGCCCAT CTTTGTTC TCATCCTAGG CTTGGAGTCA ACACAACACA  
 1251 ACAACAATGA ATTTCATT TTCTGTTCT TTACTTCCTC TTATTCCTCT  
 1301 TCCTATGTT GCCTCTTCGA CGGTGTTATT TCAGGTATCC ATCTCCAAAG  
 1351 AACCTTATTT TTCTCTTAAAC TTTCTCTATG TATATGTATC TCTATGTTA  
 1401 TGTAGTACTT GCTCAAGTAT ATAAAGAAAA GTTAGTTCT CTAGAATCTT  
 1451 TGAATTCAATT GTTGTAGGGT TCAATTGGGA TTGAGTAAT AAGCAAGGCG  
 1501 GATGGTACAA CTCTCTCATC AACTTAGTTC CGGACTTGGC TAAAGCTGGA  
 1551 GTTACTCATG TTTGGTTGCC ACCATCATCT CACTCCGTTT CTCCTCAAGG  
 1601 TAATTTCCG AGTGATTGTG ACCTAGTAAT CCAATGAAGT CAAAATAACC  
 1651 ACGGAAGATT AGAGTCTAA TTTAATGAA AATAGTTCAG ACAAGTTAAT  
 1701 GACCAACTTA TATATTAGT CAATCCATAA AATTGTGATG AGTAGTTACA  
 1751 AAATGGAATT GCTTGAAGGC TTATGCCATG TTTTATGCCA GGTTATATGC  
 1801 CAGGAAGGTT GTATGACTAG GATGCTTCCA AGTTGGAAA TCAGCAACAA  
 1851 CTGAAAATC TTATTAAGGC TTTAACATGA CCACGGATC AAAATGGTTG  
 1901 CTGATATAGT GATAATCAT AGAACTGCTG ATAACAAAGA TAGCAGGGGA  
 1951 ATATACAGCA TCTTGAAGG AGGAACATCT GATGACCGGC TTGATTGGGG  
 2001 TCCATCTTTC ATTGCAAGGA ACGACACACA ATATTCTGAT GGCACGGGGA  
 2051 ATCCAGACAC GGGTTGGAC TTTGAACCTG CACCTGATAT CGATCATCT  
 2101 AATACGAGAG TCGAGAAAGA GTTATCAGAC TGGATGAACT GGCTGAAATC  
 2151 TGAAAATTGGA TTGATGGTT GGCCTTTCGA TTTTGTAGG GGATATGCAC  
 2201 CTTGCATTAC CAAAATTAT ATGGAAACA CGTCCCCGA TTTGCTGTT  
 2251 GGTGAATTGT GGAACCTCTC TGCTTATGGC CAGGACGGGA AACCGGAATA  
 2301 TAACCAGGAC AATCATAGAA ATGAGCTAGT TGGTTGGTA AAAAATGCGG  
 2351 GGGGGCTGT AACAGCTTT GATTTACAA CAAAGGGAAAT TCTTCAAGCT  
 2401 GCAGTTCAAG AAGAGTTATG GAGATTGAAG GATCCAATG GAAAACCTCC  
 2451 TGGGATGATC GGTGTTTGC CTCGAAAGC TGTGACTTTT ATCGATAATC  
 2501 ATGATACTGG ATCGACACAA AATATGTGGC CTTTCCCTTC AGACAAAGTT  
 2551 ATGCAAGGAT ATGCATACAT TCTTACTCAT CCAGGAATCC CATCCGTGGT  
 2601 AAAAAAAATA AATAAATTCT TTCTACATAT CTCATTGT TTCTATTTAC  
 2651 AAGAAAATTAA TATTCTTTTC CAGGGGATT GAGAACTCG GCCTGTGGGA  
 2701 GTTGTGTCAC ATTGCCAGTC TCGTAATCCA TAAACAAACA CTCAAACCT  
 2751 GAGTGTGAC ACCTAGACAC CTCAACTCGT TTTTACCGT GTAAATTGAA  
 2801 CACTTCAACT TACAAAATGA TCGTGTAGCA CCTCCAAAAA TTATGTGTC  
 2851 CAATTAGCCA CGTGCAGAGT ACACGAAAAT GAGTTGGAGT AGTTAGTTGC  
 2901 CAAATAAAAC CAAGCTGAGG TGTCTAAATG TGCACTCTCA AAGTNGGATG  
 2951 TTTACTGGC AGCTGAGGCC GAGGCCATGT TTGANTGTTA TGCTTATAGG  
 3001 ATATGACACA TTGTTTCCG ATTAGCTGAG GANTTGATTA AATCCTNGTT  
 3051 TTNGTTNGCA GTTNTATNAC CATTNCCTTG ATNGGGCTN CNAGGATGGA  
 3101 ATTNCAGCAC TAANCTCTAT TAGGAAAAGG AATAGGATT GTGCANCAAG

FIGURE 10 (ii)

3151 CAATGTGCAA ATAATGGCTC CTGATTCTGA ATCTTTATAT ANCAATGGAT  
 3201 CATCACAAAA TCATTGTCAA GATTGGACCA AAACTTGATC TTGGAAATCT  
 3251 TATTCCACCT AATTATGAGG TGGCAACTTC TGGACAAGAC TATGCTGTAT  
 3301 GGGAGCAAAA GGCATAATCA TATTGTACCA CACTAAAAGG GACCATGGCC  
 3351 ACAATGGTTC TCATTAGTGT TAATGTATA TGATTGAAAA TGTAATTAT  
 3401 ATTGACATAA TGAAGGCCAA AAATTCAAGA AATTATAAAC AATTCAATAG  
 3451 TCCCTTGCTCA ATTCAACATT ACATTATGAC TTCTCTATTG CAAACTAGTT  
 3501 TGGGTCCACA TTATTGTCTC CTAaaaATTT ACAACATTC TTAAGGGAAC  
 3551 TTAATTAGTT ACAGTGAACA TATGTTGAAA TTACCCCTTA TCCCCTTACA  
 3601 ATTGATTAA TAAATATTTC CCCTATCCCT TTGGTAGTTG GTTAGAGTTA  
 3651 TAAGTAAACGT AGAGATTAGT TATAAGAGAA TTTATGTATT ATTATGCAGA  
 3701 TGTTTAGTTA TATCGATTIT AGTTATTTAT ATGTTGATT TTTCACCTTC  
 3751 AATAATGCAT ATAAAGATGG TAAATGATTG GATTGATCGA ATTCGAATGA  
 3801 GTTTGAATAT GAACTAATCT TCAAATTAA TATAAATTAA TTATTGTAG  
 3851 ATCTATAGCC AAACGGCTCC AAAACAATAA ATAATTACA TTATTGTAG  
 3901 TATTTTATT AAAATGGGAT NTTCCCTCATC CCACTTGTAC CAGTTGAAAC  
 3951 CCTAATAATA AGCCAATCCA ACCGTCAAAA TTACAAATT TGAAAATTGC  
 4001 GCTCCCTACA GTTCTCCCT ATTCAAGATTG GATTCAATTCT CTCATTTTT Dem ATG  
 4051 TGTTTCACA TTTTACCTCT AAATCAACAA AATTCCCTTT GTCAAATGG  
 4101 GTGCTTAATCA CAGCGTGAAG GATCTGGAGC TTTCTGATTG CGAGTCTGAA  
 4151 TCCGAATATG GGTCCGAGTC TCGAACAAAGG CAGGAAGAGG AAGACGAAGA  
 4201 TAACTACTCA GATGCTAAAA CGACCCCGTC TTCCACTGAT CGGAAACAGA  
 4251 GCAAAACCCC GTCTCTTTG GATGATGTTG AAGCAAAGCT GAAAGCTTTA  
 4301 AAGCTTAAGT ATGGTACTCC TCATGCTAAA ACCCCCACAG CGAAAAACCC  
 4351 TGTTAAACTT TACCTTCATG TTGGTGGGAA CACTGGAAT TCCAAATGGG  
 4401 TAGTTTCATG TAAGGTGACA GCTTATTCGT TGTTAAATC GGTTAGTGAG  
 4451 GATGGATGG ATGATGATGA AAATGAAAGAA ACTGAGGAGA ATGCTTGGTG  
 4501 GGTTTGAAA ATTGGGTGAA AGGTTGGGAA TAAGATTGAT GAGAAATTG  
 4551 AGCTCAAGGC ATTTAAGGAG CAGAAAAGGG TGGATTTTGT GGCGAAATGGG  
 4601 GTTTGGGCTG TGAGATTCTT TGGGGAGGAA GAGTATAAGG CGTTCAATTGA  
 4651 CTTATATCAG AGCTGTTGT TTGAGAAATAC TTATGGTTT GAGGCAAATG  
 4701 ATGAGAAATAG AGTTAAGGTG TATGGTAAAG ACTTTATGGG GTGGGCAAAT  
 4751 CCAGAAGCTG CGGATGATTG AATGTTGGAG GATGCTGGGG ATAGCTTCGC  
 4801 GAAGAGCCCT GCGTCTGAAA AGAAGACACC TTGAGGGTT AACCATGATT  
 4851 TGAGGGAGGA GTTGTGAGGAG CCAGCTAAAG GAGGAGCTAT TCAGAGCTTG  
 4901 GCATTAGGTG CGTTGGATAA TAGTTTCTT ATAAGTGTGATT CTGGAAATTCA  
 4951 GGTGAGGAGG AACTATACTC ATGAAATAAG TGGAAAGGT GTTGTGTCA  
 5001 ATTGTTGATAA GGAAAGGTCT GCTGTACCTA ATTCAACTCC AAGGAAAGCT  
 5051 CTACTTCTAA GAGCAGAGAC TAATATGCTT CTCACTGAGTC CAGTGACTGA  
 5101 TAGAAAGCT CACTCTCGGG GATTACATCA GTTGTGATATC GAGACTGGG  
 5151 AGGTGTTAG CGAGTGGAAAG TTGAGAAAG ATGAAACTGCA TATCACGATG  
 5201 AGGGATATCA CTAATGATAG CAAAGGAGCT CAGATGGATC CTCGGGGTC  
 5251 TACTTCTTA GGGCTAGATG ATAACAGATT GTGTAGGTG CATAATGGCTG  
 5301 ATCGGCATGG GATGGTCCAG AATCTAGTTG ATGAAAGTAC TCTGTGCTG  
 5351 AATTGGACTC AAGGACATCA ATTTCGAGG GGAACAACT TTCACTGCTT  
 5401 TGCTACTACT GGTGATGGAT CAAATGTGTGTT GATGGCAAGA  
 5451 TTAGATGTTA CTCACAGCT TCCATGAGAC AGGCTAAAAC TGCTTTTCCA  
 5501 GGCCTAGGT CTCCTATCAC TCATGTTGGAT GTTACCTATG ATGGGAAGTG  
 5551 GATATTGGGG ACAACTGATA CTTACTTGTAT ATTGATATGC ACCTTGTAA  
 5601 TCGACAAAGAA TGGAAACTACT AAGACTGGTT TTGCTGGTCG CATGGGAAAT  
 5651 AAGATTTCGG CTCCAAGATT GTTAAAGCTA AACCTCTCG ATTACATAT  
 5701 GGCTGGAGCT AACAAAGTTCG CCAGTGCTCA ATTTCATGG GTCACCGAGA  
 5751 ATGGGAAGCA AGAGGCCAC CTGGTGTGCTA CTGTTGGAA GTTGTAGGTG  
 5801 ATCTGGAAATT TICAACAGGT GAGGGATGGT TCTCATGAGT GTTACCAAGA  
 5851 TCAGGTGGGG TTGAAAGAGCT GCTTATGTTA CAAGATAGTC CTAAGAGACG  
 5901 ACTCTATTTGT AGAAAGTCGT TTCACTGATG ACAAGTACGC TGTGTTCTGAC  
 5951 TCACCTGAAAG CACCACTGGC GGTAGGCAACC CCCATGAAAG TCAGCTCATT  
 6001 CAGCACTCTC AGCAAGGGCT TACAAATTG AACAACTATT CTGTTCTATAT  
 6051 ACGCAACTTA TTGATTAT CTCAGGAGA ATTAGTGCT CTCACACTAA

## FIGURE 10 (iii)

6101 GTAGCTTGAA AAACCTGCACA TCTGCAGATC ATTTCCAGTT CAAATGTATTA  
6151 CTACTTTAGT TTAAAAACCT TAAAAGGCAG TCTTCCAAAT TCTAGGTATC  
6201 CTCACCTGAC ATTATTATTG TTGTAATAGC TAATTGTTGC TTGCTCTAAA  
6251 TCCCCGTTCA ATG

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**FIGURE 11**

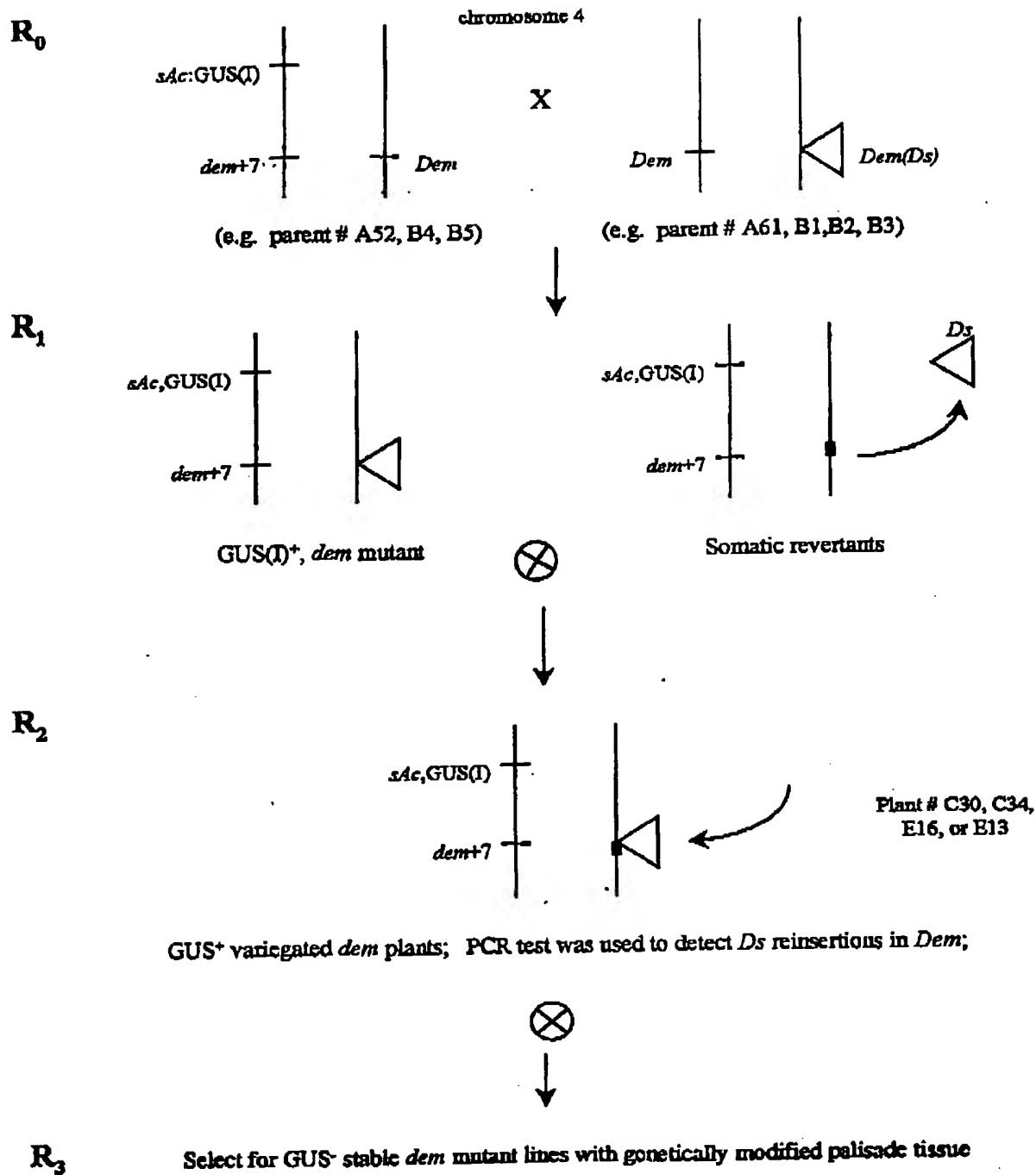


FIGURE 12

FIGURE 13

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